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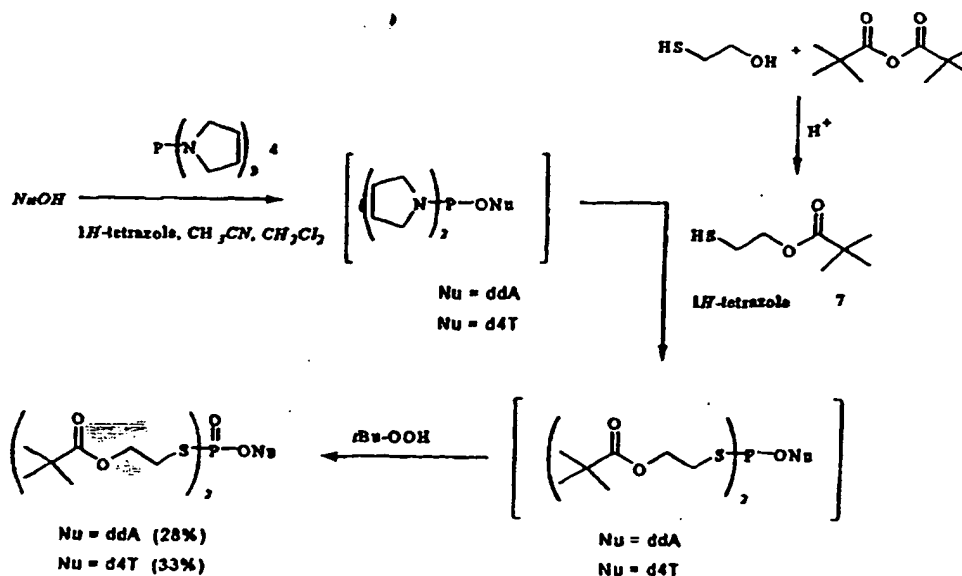
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(54) Title: **BIOLOGICALLY ACTIVE PHOSPHOTRIESTER-TYPE COMPOUNDS**



(57) Abstract

Compounds of formula RS-P(=O)(QR)-Nu where: R is a radical $-(\text{CH}_2)_n\text{-W-X}$; X is a radical $-\text{C(=Z)}$ (Y) or $-\text{S-U}$; Z is O or S; W is O or S; Q is O or S; Y and U are an alkyl, aryl or saccharide radical which is optionally substituted with, for example, an OH, SH or NH group; n is equal to 1 to 4, preferably 1 or 2; and Nu is a radical consisting of a residue of a biologically active compound or the dephosphorylated residue of a compound which is biologically active when it bears a phosphate or phosphonate group.

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BIOLOGICALLY ACTIVE PHOSPHOTRIESTER-TYPE COMPOUNDS

RELATED APPLICATION

This patent application is a continuation-in-part of application Serial No. 08/416,515, filed April 4, 1995, the
5 entire contents of which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to the bioreversible functionalization of phosphate or phosphonate groups of biologically active compounds.

10 The present invention relates more particularly to phosphotriester-type biologically active compounds bearing phosphate or phosphonate groups which are protected by protecting groups that are bioreversible in an intracellular medium.

15 BACKGROUND OF THE INVENTION

Compounds bearing a phosphate or phosphonate group have a negatively charged ionic nature and a physiological pH. As a result, the therapeutic activity of such compounds is limited by the low diffusion of negatively charged compounds
20 across biological lipid membranes. Moreover, compounds bearing phosphate groups are readily dephosphorylated by the action of phosphatase enzymes in the blood or on cell membranes, which enzymes dephosphorylate substrate compounds. In general, charged phosphate or phosphonate compounds are poorly absorbed

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via oral administration, and do not diffuse efficiently across cell membranes or even the cerebral barrier, which are lipidic in nature.

Certain compounds, such as nucleoside derivatives or
5 analogs, are active agents that are administered in non-phosphorylated form, but are phosphorylated in vivo in the form of metabolic monophosphate or triphosphate to become active.

Thus, nucleoside derivatives having antitumor activity, such as 5-fluorouridine, 5-fluoro-2'-deoxyuridine or
10 1-O-D-arabinofuranosylcytosine, exert their activity in phosphorylated form.

Similarly, in order to exert their antiproliferative activity, certain nucleoside or phosphononucleoside analogs need to be phosphorylated into the corresponding triphosphate
15 thereof by cellular or viral enzymes; this triphosphate is then capable of inhibiting the viral and/or cellular polymerases.

Among the various structural classes of antiviral agents, 2', 3'-dideoxynucleosides are among the most effective compounds in the treatment of AIDS. However, these nucleoside
20 analogs must undergo a biotransformation by cell kinases in order to exert their activity on the replication of HIV, the etiological agent of AIDS. This metabolization occurs via the dideoxynucleoside 5'-monophosphate and then the 5'-diphosphate to lead to the 5'-triphosphate, which is an inhibitor of HIV
25 reverse transcriptase and which thereby interferes with the biosynthesis of viral DNA.

Despite their great therapeutic potential, 2',3' - dideoxynucleosides suffer from limitations, in particular the low metabolizability of some of them by kinases into
30 triphosphate. 2',3'-Dideoxyuridine 5'-triphosphate, for example, is an excellent inhibitor of reverse transcriptase (Z. Hao et al., Proc. Am. Assoc. Cancer Res., 1988, 29, 348, E. Matthes et al., Biochem. Biophys. Res. Commun, 1987, 148, 78-85). However, the nucleoside thereof is able to inhibit the
35 replication of HIV in vitro. Studies have shown that this result is linked to the low metabolizability of the nucleoside

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into its monophosphate by cell kinases (Z. Hao et al. Mol. Pharmacol. 1990, 37, 157-153).

Thus, AZT is successively metabolized into the triphosphate thereof (AZTP), which is a potent inhibitor of HIV
5 reverse transcriptase. Similarly, Acyclovir (ACV) is converted into the triphosphate thereof (ACVTP), which selectively inhibits herpesvirus DNA polymerase. The first step in the activation of the nucleosides (Nu) consists of a monophosphorylation, leading to the corresponding monophosphate
10 (NUMP). It is this first step which is the most selective.

In order to circumvent this key step of enzymatic monophosphorylation, it has already been proposed to administer NuMPs directly, but their use for therapeutic purposes was contraried by the abovementioned limitations and drawbacks.

15 Compounds bearing a phosphate or phosphonate group have a negatively charged ionic nature at physiological pH. The therapeutic activity of such compounds is consequently limited, on account of the low diffusion of negatively charged compounds across biological lipid membranes. In particular,
20 charged compounds do not diffuse efficiently across cell membranes, or indeed across the cerebral barrier, which are lipidic in nature. Moreover, such compounds are readily dephosphorylated by the action of phosphatase enzymes in the blood or on the cell membranes, which enzymes dephosphorylate
25 the substrate compounds thereof. In general, charged phosphate or phosphonate compounds are poorly absorbed via oral administration.

It has been sought to convert mononucleotides into neutral phosphotriesters capable of crossing the cell membrane
30 and of intracellular delivery of the corresponding mononucleotide phosphotriester (NUMP). Such an approach has been adopted by various authors for a number of years, but has proved to be disappointing. The derivatives obtained were in general either excessively toxic or of insufficient
35 extracellular stability, and did not in the end result provide any enhancement of the biological activity.

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Thus, the use of phosphorylated nucleoside structures comprising bioreversible protecting groups of acyloxymethyl or acyloxybenzyl type has been proposed, for antitumor nucleoside derivatives such as 5-fluorouracil, in WO patents No. 9,008,155
5 and 9,119,721. However, these compounds are of limited chemical stability, and generate toxic formaldehyde metabolites in vivo. Furthermore, they are sparingly soluble and the yield of their chemical preparation is low.

The aim of the present invention is thus to provide
10 other types of bioreversible groups which may be combined especially with mononucleotide or other structures such that the biological activity thereof is enhanced, in particular as regards compounds derived from or analogous to nucleosides having antiviral activity, and which reversible groups do not
15 have the abovementioned drawbacks.

The present invention proposes to use novel groups, characterized by the presence of -SIS- and/or -S/C=Z enzyme-labile bonds which lead, after enzymatic activation, to the formation of unstable intermediates that selectively release
20 the corresponding monophosphate or monophosphonate.

More precisely, the subject of the present invention is the compound corresponding to the general formula I:



in which:

25 R is a radical $-(\text{CH}_2)_n\text{-S-X}$, where X represents a radical $-\text{C(=Z)(Y)}$ or $-\text{S-U}$, and Z is O or S;

Y and U represent an alkyl, aryl or saccharide radical which is optionally substituted, in particular with an OH, SH or NH group; and

30 n is equal to 1 to 4, preferably 1 or 2;

Nu is a radical consisting of a residue of a biologically active compound or the dephosphorylated residue of a compound which is biologically active when it bears a phosphate or phosphonate group.

35 Moreover, the present invention also relates to the compound corresponding to the general formula Ia:



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in which:

R is a radical $-(CH_2)_n-W-X$, where X represents a radical $-C(=Z)(Y)$ or $-S-U$, and Z is O or S;

Q is O or S;

5 W is O or S;

Y and U represent an alkyl, aryl or saccharide radical which is optionally substituted, in particular with an OH, SH or NH group;

n is equal to 1 to 4, preferably 1 or 2; and

10 Nu is a radical consisting of a residue of a biologically active compound or the dephosphorylated residue of a compound which is biologically active when it bears a phosphate or phosphonate group.

When, in the formulas (I) and (Ia), Nu is linked to
15 the phosphorus by a P-O bond, the compound of formulas (I) and (Ia) according to the invention bears a phosphate group and thus constitutes a phosphotriester compound.

When Nu is linked to the phosphorus by a P-C bond,
the compound of formulas (I) and (Ia) according to the
20 invention bears a phosphonate group.

The mechanisms of bioreversibility of the radicals R take place via enzymatic cleavage of the S-X or O-X bonds and release of the $(CH_2)_2-S$ residues, according to the mechanisms which are illustrated by the examples represented Figure 1 and
25 Figure 9.

For Y and U there are especially mentioned, as alkyl group, a C_1 to C_4 alkyl; as aryl group, phenyl and benzyl radicals, and, as saccharide radicals, glucose, mannose or rhamnose.

30 In one embodiment, when X represents SU, U preferably represents the radical $-(CH_2)_{n^1}-X^1$ where X^1 represents H, OH, SH or NH_2 and n^1 is equal to 1 to 4, preferably 1 or 2.

There are especially mentioned the compounds (I) and (Ia) in which R represents $-(CH_2)_2-S-S-(CH_2)_2-OH$.

35 In another embodiment, when X represents $-C(=Z)Y$, Y appropriately represents CH_3 or tBu.

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There are especially mentioned the compounds (I) and (Ia) for which R represents $-(CH_2)_n-S-C(=O)-CH_3$ or $(CH_2)_n-S-C(=O)-tBu$ with $n = 1$ or 2 .

In an advantageous embodiment of the present invention, for the compounds (I) and (Ia), there are especially mentioned the compounds for which Nu represents a 5' residue of a natural nucleoside or of a derivative of a natural nucleoside, which is therapeutically active or for which the 5'-(O)-monophosphate or 5'-(C)-monophosphonate is therapeutically active.

These compounds of formulas (I) and (Ia) generally have antiviral or antitumor activity.

The compounds of formulas (I) and (Ia) for which Nu represents a 5' residue of 2',3'-dideoxynucleoside or 2',3'-didehydronucleoside are more particularly mentioned.

The compounds (I) and (Ia) for which Nu is a 5' residue of ddU (dideoxyuridine), ddT (dideoxythymidine), ddC (dideoxycytidine), AZT (3'-azido-2',3'-dideoxythymidine) and the derivatives thereof, especially those substituted on the pyrimidine base or at 2' and 3' of the saccharide ring, are more particularly mentioned among the compounds (I) and (Ia) derived from dideoxynucleosides having antiviral activity.

ddT, ddC or AZT are illustrations of the radicals Nu which represent a 5' residue of a therapeutically active natural nucleoside derivative.

ddU is an illustration of the radicals Nu which represent a 5' residue of a nucleoside derivative which is only active in phosphorylated form. ddU (dideoxyuridine) is not enzymatically monophosphorylated in vivo. Only the triphosphate thereof is a polymerase inhibitor and imparts antiviral activity thereto.

The compounds for which Nu represents a 5' residue of the derivatives 5-fluorouridine or 5-fluoro-2'-deoxyuridine or 1- β -D-arabinofuranosylcytosine are especially mentioned among the compounds (I) and (Ia) having antitumor activity. These compounds illustrate the advantage of the functionalization according to the invention in order to

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circumvent the resistance acquired to certain nucleoside drugs when this resistance is due to a loss of their ability to be monophosphorylated, as is often the case in antitumor chemotherapy.

5 According to another embodiment variant of the invention, in the compounds (I) and (Ia) the radical Nu represents a nucleoside analog residue such as a carbonucleoside (nucleoside in which the oxygen of the saccharide ring is replaced by a carbon), a phosphononucleoside
10 (nucleoside in which the oxygen at 5' is replaced by a carbon) or a purine- or pyrimidine-based derivative of acyclonucleoside type, that is to say one which contains no saccharide ring, such as ACV (aciclovir), or a methoxyalkylpurine or pyrimidine radical of formula $\text{CH}_2\text{-O-alkylpurine}$ or -pyrimidine .

15 The compounds (I) and (Ia) for which Nu represents a methoxyalkylpurine or -pyrimidine radical are illustrations of the phosphonate compounds. In the particular case of phosphonylmethoxyalkylpurine or -pyrimidine antiviral compounds, PMEA, HMPA or HPMPC are especially mentioned, the
20 formulae of which are given in Figures 3 and 4.

Thus, the present invention relates in particular to compounds in which Nu is a 3-hydroxy-2-methoxypropylpurine or -pyrimidine radical of formula: $\text{-CH}_2\text{-OCH(CH}_2\text{OH)-CH}_2\text{-purine}$ or -pyrimidine or a 2-methoxyethylpurine or -pyrimidine radical of
25 formula $\text{-CH}_2\text{-O-C}_2\text{H}_4\text{-pyrimidine}$ and, for example, the compounds (I) and (Ia) for which Nu is a methoxyethyladenine or 3-hydroxy-2-methoxypropylcytosine radical.

When Nu represents a dephosphonylated residue (dephosphated or dephosphonated) of a molecule which is
30 biologically active when it is in phosphate or phosphonate form, the functionalization according to the invention may enable the physicochemical and biophysical parameters of the said molecule comprising a phosphate or phosphonate group to be modified in general. Compounds (I) and (Ia) may then consist, for example,
35 of a phosphopeptide or phospholipid compound.

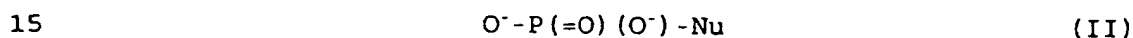
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When Nu represents a residue of a nucleoside, of a nucleoside derivative or of a nucleoside analog, the latter may be D or L enantiomers.

The compounds according to the invention may be prepared by processes known to those skilled in the art.

In particular, the subject of the present invention is a process for the preparation of the compounds according to the invention, characterized in that a compound of formulas (I) and (Ia) is prepared, in which compound the functional groups of R, and possibly of Nu, are protected by suitable protecting groups, followed by deprotection of the said functional groups of R, and possibly of Nu, in order to obtain the compounds of formula (I) and (Ia).

In particular, a compound of formula (II):



where Nu is possibly protected, is reacted in an appropriate manner with the compound of formula (III):



where X is protected, in order to obtain the said protected compound of formula (I), which is then deprotected.

In a particular embodiment, the reaction between the compounds of formula (II) and (III) takes place in the presence of a condensing agent such as MSNT, in pyridine.

Other preparation processes are illustrated in the examples which follow, in which other characteristics and advantages of the present invention will also appear.

BRIEF DESCRIPTION OF THE DRAWINGS

The numerous objects and advantages of the present invention may be better understood by those skilled in the art by reference to the accompanying figures, in which:

Figure 1 represents decomposition mechanisms for groups which are bioreversible under enzymatic activation. The same mechanism takes place for both groups R.

Figure 2 represents the decomposition mechanism for the bioreversible group of the compound of Example 2.

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Figure 3 represents the formula of certain compounds according to the invention.

Figure 4 represents a preparation scheme for compounds prepared in Example 1, and the formula of the
5 compounds HMPMA and HPMPC.

Figure 5 represents the preparation schemes for compounds prepared in Examples 2 and 3.

Figure 6 represents the preparation scheme for compounds prepared in Example 4.

10 Figure 7 represents the preparation scheme for compounds prepared in Examples 6-14.

Figure 8 represents the preparation scheme for compounds prepared in Examples 15 and 16.

15 Figure 9 represents the decomposition mechanism for the bioreversible group of the compound of Example 16A.

Figure 10 illustrates anti HIV activity in cell cultures comparing compounds of Example 16A and 16B with similar compounds of formula I.

DETAILED DESCRIPTION OF THE INVENTION

20 The advantage of this invention resides in the difference in stability of the mononucleotide phosphotriesters between extracellular and intracellular media; it is initially shown that the decomposition of one of the compounds described in the invention (Example 2) complies fully with the
25 abovementioned criteria and occurs according to the mechanism shown Figure 2.

The "ISRP on line" HPLC technique ("On-line Internal Surface Reversed-Phase Cleaning: The Direct HPLC Analysis of Crude Biological Samples", A. Pompon, I. Lefebvre and J.L.
30 Imbach, Biochemical Pharmacology, 43,1769-1775 (1992) was used for this study, the compound studied being incubated respectively in culture medium (RPMI/10% inactivated serum) and in a total cell extract (CEM).

The compound of Example 2 has a half-life of 9 hours
35 in culture medium and of less than 5 minutes in cell extract. The corresponding intracellular release of NUMP is corroborated

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by the demonstration of biological activity, whereas the constituent nucleoside is inactive.

Furthermore, insofar as the rate-determining step for activation of the phosphotriester into mononucleotide is highly
5 dependent on the initial kinetics of enzyme hydrolysis, a variation in the nature of the enzymelabile groups leads to a modulation of the pharmacokinetic parameters of the drug and results in delayactions.

These data clearly confirm the advantage of the
10 invention.

Thin layer chromatographies were performed on Merck 60F 254 silica plates (Art. 5554). Column chromatographies on silica gel were carried out with Merck 60 H silica (Art. 7736) or with RP2 Merck silanized silica (Art. 7719). Before
15 analysis or lyophilization, the solutions were filtered on Millex HV-4 filter (Millipore).

The UV spectra were recorded on a UVIKON 810 spectrophotometer.

Mass spectra were taken on a JEOL JMS DX 300
20 apparatus by the FAB ionization method in positive or negative mode in a matrix of glycerol (GT), glycerol/thioglycerol (GT) or 3-nitrobenzyl alcohol (NBA).

Proton NMR spectra were recorded on a Varian EM 360 apparatus or on a Bruker AC 250 apparatus. The chemical shifts
25 are expressed in ppm relative to the tetramethylsilane (TMS) signal. The multiplicity and the appearance of the signals observed by NMR are indicated by one (or more) letter(s): s (singlet), d (doublet), t (triplet), m (multiplet), b (broad). Phosphorus NMR spectra were recorded on a Bruker WP 200 SY
30 apparatus with proton decoupling. The chemical shifts are expressed in ppm relative to the H_3PO_4 signal which is taken as external reference.

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Example 1:

O-(2',3'-dideoxyuridin-5'-yl)-O,O'-bis(S-acetyl-2-thioethyl)phosphate (1) (Scheme in Figure 4)

2-Hydroxyethyl acetyl sulfide (5)

5 A solution of 1.0 ml (14 mmol) of thioacetic acid in 5 ml of toluene is treated with 0.90 ml (12 mmol) of iodoethanol in the presence of 1.7 ml (12 mmol) of 1,8-diazabicyclo-(5.4.0)-7-undecene (DBU) for 2 hours. The reaction medium is diluted with dichloromethane and washed with
10 water. The organic phase is dried over sodium sulfate and evaporated. The crude product obtained is purified on a column of silica gel (eluent: methanol (0-4%) in dichloromethane) to give 1.2 g (85%) of 5 in the form of an oil.

5 : ¹H NMR (DMSO-d₆) : δ = 2.32 (s, 3H, CH₃); 2.91 (t, 2H, CH₂S, J = 6.6 Hz); 3.45 (pseudo q, 2H, CH₂OH, J = 6 Hz);
15 4.97 (t, 1H, OH) ppm.

O-(2',3'-dideoxyuridin-5'-yl) hydrogenophosphonate (6)

A 1.5 M solution of phosphorous acid (165 ml, 247
20 mmol) in anhydrous pyridine is added to 5.25 g of 2',3'-dideoxy-uridine (24.7 mmol) and is treated with 16.8 ml of pivaloyl chloride (136 mmol). After reaction for 3 hours, aqueous 1 M triethylammonium bicarbonate solution is added to neutralize the mixture and the solvent is evaporated off under
25 reduced pressure. The oil obtained is chromatographed on a column of silica gel (eluent: methanol (0-35%) in dichloromethane) to give 6. The product is taken up in methanol and is filtered on a Millipore filter. Evaporation of the solvent gives 7.10 g (76%) of 6 (in triethylammonium
30 form) which is sufficiently pure for use in the next step of the synthesis. A sample of higher purity is obtained after an additional purification by thin layer chromatography on silica gel, using a mixture of isopropanol, ammonia solution and water (8:1:1) as eluent. The product, in ammonium form, is extracted
35 from the silica with methanol, the solvent is stripped off by evaporation and the residue is taken up in water, filtered on a Millipore filter and lyophilized.

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6 : LTV (H₂O): Λ_{\max} = 262 nm (e 9940); Λ_{\min} = 230 nm (e 2080)

MS (negative FAB, GT); 275 (M)⁻

¹H NMR (DMSO-d₆): d = 1.78-2.05 (m, 3H, H-2', 3', 3");
5 2.18-2.45 (m, 1H, H-2"); 3.65-3.95 (m, 2H, H-5', 5"); 4.11 (m, 1H, H-4"); 5.55 (d, 1H, H-5, J = 8.1 Hz); 5.95 (dd, 1H, H-1', J = 6.8 and 3.8 Hz); 6.63 (d, 1H, HP, J = 592 Hz); 7.87 (d, 1H, H-6, J = 8.1 Hz) ppm
31 P NMR (DMSO-d₆): d = 1.60 ppm.

O-(2',3'-dideoxyuridin-5'-yl)-O,O'-bis(S-acetyl-2-
10 thioethyl)phosphate (1)

A solution of 200 mg (0.530 mmol) of the hydrogenophosphonate 6 of 2',3'-dideoxyuridine in 5 ml of pyridine is treated with 196 μ l of pivaloyl chloride for 30 minutes. 159 mg (1.33 mmol) of 2-hydroxyethyl acetyl sulfide (5) are added
15 and the reaction is left stirring for 2 hours. The phosphate formed is oxidized using 2% iodine solution in a pyridine-water mixture (98:2) until a persistent coloration is obtained (7-8 ml). The solvent is evaporated off under reduced pressure. The crude product obtained is co-evaporated with toluene and
20 chromatographed on a column of silica gel (eluent: methanol (0-6%) in dichloromethane) to give 65 mg (25%) of compound 1 in the form of an oil.

1: UV (EtOH): Λ_{\max} = 262 nm (e 9400); Λ_{\min} = 230 nm (e 2500)

25 MS (positive F.A.B): 497 (M+H)⁺

¹H NMR (DMSO-d₆): d = 1.73-2.13 (m, 3H, H-2', 3', 3")
2.20-2.4 (m, 1H, H-2"); 2.356 and 2.360 (s and s, 3H and 3 H.
2 CH₃); 3.13 (t, 4H, 2 CH₂S, J = 6.4 Hz) ; 4.00 - 4.26 (m, 7H, H-4', 5', 5" and 2 CH₂CH₂OP); 5.60 (d, 1H, H-5, J = 8.1 Hz);
30 6.01 (dd, 1H, H-1', J = 4.2 and 7.0 Hz); 7.64 (d, 1H, H-6, J=8.1 Hz); 11.3 (bs, 1H, NHCO) ppm.

³¹P NMR (DMSO-d₆): d = -1.21 ppm

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Example 2:

O,O'-Bis(S-(2-hydroxyethylsulfidyl)-2-thioethyl)-O-(2',3'-dideoxyuridin-5'-yl)phosphate (2). (Scheme in Figure 5)

O,O'-Bis(S-(O-(4-methoxytrityl)-2-oxethylsufidyl)-2-thioethyl)phosphate (8).

To a solution of 0.910 g (13.4 mmol) of imidazole in 18 ml of pyridine at 0C is added 0.406 ml (4.45 mmol) of phosphorus oxychloride. The mixture is stirred for 30 minutes at room temperature, then added to 3.80 g (8.91mmol) of mono-O-(4-methoxytrityl)dithiodiethanol (7). After 18 hours, the reaction mixture is treated with 1 M triethylammonium acetate solution. The reaction products are extracted with dichloromethane and the organic phase is washed with water, dried over sodium sulfate, concentrated under reduced pressure and co-evaporated with toluene. Purification on a column of silica gel (eluent: methanol (0-10%) in dichloromethane) gives 2.2 g (48%) of 8 in the form of the triethyl-ammonium salt.

8: MS (negative FAB, NBA): 913 (M⁻).

¹H NMR (DMSO-d₆) 1.14 (t, 9H, (CH₃CH₂)₃NH⁺ J = 7.3 Hz); 2.78 (t, 4H, 2 SCH₂CH₂OP, J = 6.4 Hz); 2.86 (t, 4H, 2 SCH₂CH₂OMTr, J = 6 Hz) ; 2.99 (q, 6H, (CH₃CH₂)₃NH⁺, J = 7.3 Hz); 3.21 (t, 4H, 2 CH₂OMTr, J = 5.9 Hz); 3.71 (s, 6H, 2 CH₃O); 3.87 (m, 4H, 2 CH₂OP); 6.82-7.45 (m, 28H, 2 Tr) ppm.

³¹P NMR (DMSO-d₆): -2.70 ppm.

O,O'-Bis(S-(2-hydroxyethylsufidyl)-2-thioethyl)-O-(2',3'-dideoxyuridin-5'-yl)phosphate (2).

A mixture of 666 mg (0.655 mmol) of 8 and 139 mg (0.656 mmol) of 2',3'-dideoxyuridine in 5 ml of pyridine is treated with 486 mg (1.64 nmol) of 1-(2-mesitylene-sulfonyl)-3-nitro-1,2,4-triazole. After 30 hours, the reaction mixture is diluted with dichloromethane and washed with aqueous 1M triethylammonium acetate solution and then with water. The organic phase is dried over sodium sulfate, concentrated under reduced pressure, coevaporated with toluene and chromatographed on a column of silica gel (eluent: methanol (0-4%) in dichloromethane). The partially purified protected

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phosphotriester is treated with 5 ml of the acetic acid/water/methanol mixture (8:1:1) for 24 hours. The solvents are stripped off by evaporation under reduced pressure and the oil obtained is co-evaporated with toluene. Purification on a
 5 column of silica gel (eluent: methanol (0-6%) in dichloromethane) followed by purification on a column of silanized silica (eluent: ethanol (0-40%) in water) gives 52 mg (14%) of compound 2 after lyophilization in dioxane.

2: LTV (EtOH): λ_{\max} 261 nm (ϵ 9900); λ_{\min} 231 nm (ϵ
 10 3100)

MS (positive F.A.B, GT) : 565 (M+H)⁺; 489 (M-SCH₂CH₂OH+2H)⁺; 429 (M-HOCH₂CH₂SSCH₂CH₂+2H)⁺.

¹H NMR (DMSO-d₆): 1.63-1.9 (m, 1H, H-3'); 1.9-2.10 (m, 2H, H-2'3"); 2.33-2.40 (m, 1H, H-2"); 2.80 (t, 2H, HOCH₂CH₂S, J = 6.4 Hz); 2.81 (t, 2H, HOCH₂CH₂S, J = 6.4 Hz)
 15 ; 3.00 (t, 4H, 2 SCH₂CH₂OP, J = 6.3 Hz); 3.61 (pseudo q, 4H, 2, HOCH₂, J = 6 Hz), 4.07-4.32 (m, 7H, H-4', 5', 5" and 2 CH₂CH₂OP); 4.89 (t, 2H, 2 HO, J = 4.9 Hz); 5.598 (d, 1H, H-5, J = 8.1 Hz); 5.604 (d, 1H, H-5, J = 8.1 Hz); 6.00 (dd, 2H, 2H-
 20 1', J = 4.1 and 7.9 Hz); 7.65 (d, 2H, 2 H-6, J = 8.0 Hz); 11.31 (bs, 1 H, NHCO)ppm.

³¹P NMR (DMSO-d₆): -0.880 ppm

Example 3:

O,O'-Bis(S-(2-hydroxyethylsufidyl)-2-thioethyl)-O-(3'-azido-3'-
 25 deoxythymidin-5'-yl)phosphate (3). (Scheme in Figure 5)

A mixture of 666 mg (0.655 mmol) of 8 and 193 mg (0.722 mmol) of 3'-azido-3'-deoxythymidine in 5 ml of pyridine is treated with 486 mg (1.64 mmol) of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole. After 24 hours, 194 mg (0.656 mmol)
 30 of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole are added and the reaction is left for a further 24 hours. The reaction mixture is then diluted with dichloromethane and washed with aqueous 1M triethylammonium acetate solution and then with water. The organic phase is dried over sodium sulfate, concen-
 35 trated under reduced pressure, co-evaporated with toluene and chromatographed on a column of silica gel (eluent: methanol (0-

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2%) in dichloromethane). The partially purified protected phosphotriester is treated with 5 ml of the acetic acid/water/methanol mixture (8:1:1) for 24 hours. The solvents are stripped off by evaporation under reduced pressure and the
 5 oil obtained is co-evaporated with toluene. Purification on a column of silica gel (eluent: methanol (0-6%) in dichloromethane) gives 130 mg (29%) of compound 3 after lyophilization in dioxane.

3: UV (EtOH): λ max 264 nm (ϵ 9600), λ min 234 nm (ϵ
 10 2100)

MS (positive FAB, GT): 620 (M+H)⁺; 544 (M-SCH₂CH₂OH+2H)⁺

¹H NMR (DMSO-d₆): 1.80 (s, 3H, CH₃); 2.26-2.5 (m, 2H, H-2' 2"); 2.796 (t, 2H, HOCH₂CH₂S, J = 6.4 Hz); 2.802 (t,
 15 2H, HOCH₂CH₂S, J = 6.4 Hz); 2.99 (t, 4H, 2 SCH₂CH₂OP, J = 6.3 Hz); 3.61 (pseudo q, 4H, 2 HOCH₂' J = 6 Hz); 4.02 (m, 1H, H-4'); 4.09-4.44 (m, 6H, H-5', 5" and 2 CH₂CH₂OP); 4.48 (m, 1H, H-3'); 4.90 (t, 2H, 2 HO, J = 5.3 Hz); 6.14 (t, 1H, H-1', J = 6.6 Hz); 7.49 (s, 1H, H-6); 11.37 (bs, 1H, NHCO) ppm.

20 ³¹P NMR (DMSO-d₆): -0.954 ppm

Example 4:

9-(2-(O,O'-Bis(S-(2-hydroxyethylsufidyl)-2-thioethyl)-phosphonylmethoxy-ethyl)adenine (4). (Scheme in Figure 5)

N6 -(4 - Methoxytrityl) - 9 - (2 -
 25 diethoxyphosphonylmethoxy ethyl)adenine (10).

A solution of 3.93 g (11.9 mmol) of 9-(diethoxyphosphonylmethoxyethyl)adenine (9) (A. Holy et al., Collection Czechoslovak Chem. Commun. 52 2792, 1987) and 146 mg (1.19 mmol) of 4-dimethylaminopyridine in 50 ml of
 30 dichloromethane is treated with 3.31 ml (23.8 mmol) of triethylamine and 7.35 g (23.8 mmol) of 4-methoxytrityl chloride for 4 hours. The reaction mixture is then diluted with dichloromethane and washed with aqueous sodium hydrogen carbonate solution and then with water.

35 The organic phase is dried over sodium sulfate and concentrated under reduced pressure. Chromatography on a

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column of silica gel (eluent: methanol (0-3%) in dichloromethane) allows 5.43 g (84%) of compound 10 to be isolated.

10: UV (EtOH): Λ_{\max} 275 nm (ϵ 27200), Λ_{\min} 246 nm (ϵ 11200)

MS (negative FAB, GT) 601 (M-H)⁻; 406 (A^oTr)⁻; 328 (M-MTr) -

¹H NMR (DMSO-d₆): 1.10 (t, 6H, 2 CH₃CH₂, J = 7.0 Hz); 3.71 (s, 3H, CH₃O), 3.80-3.98 (m, 4H, PCH₂ and CH₂CH₂); 3.88 (q, 4H, 2 CH₃CH₂, J = 8 Hz); 4.33 (t, CH₂CH₂, J = 4.8 Hz); 6.80-7.37 (m, 14H, Tr); 7.91 (s, 1H, H-8); 8.18 (s, H-2) ppm.

³¹P NMR (DMSO-d₆): 21.35 ppm.

N⁶-(4-Methoxytrityl)-9-(2-phosphonylmethoxy-ethyl)adenine (11).

A solution of 5.00 g (8.31 mmol) of 10 in 29 ml of acetonitrile is treated with 3.29 ml (24.9 mmol) of trimethylsilyl bromide for 14 hours. The excess reagent and the solvent are stripped off by evaporation under reduced pressure. The oil obtained is taken up in triethylanmonium bicarbonate and concentrated under reduced pressure. Purification is performed by chromatography on a column of silica gel (eluent: methanol (0-50%) in dichloromethane). After filtration in solution in di-chloromethane, 3.4 g (63%) of 11 are isolated in the form of a mixed salt of acid and triethylanmonium (1:1).

11: MS (negative FAB, GT) : 544 (M-H)⁻; 272 (M-MTr)⁻.

¹H NMR (DMSO-d₆): 1.11 (t, 9H, (CH₃CH₂)NH⁺, J = 7.3 Hz); 2.96 (q, 6H, (CH₃CH₂)NH⁺, J = 7.3 Hz); 3.34 (d, 2H, PCH₂, J = 8.4 Hz); 3.68 (s, 3H, CH₃O); 3.8 (m, 2H, CH₂CH₂); 4.27 (t, CH₂CH₂, J = 4.5 Hz); 6.65 - 7.35 (m, 14H, Tr); 7.83 (s, 1H, H-8); 8.31 (s, 1H, H-2) ppm.

³¹P NMR (DMSO-d₆): 11.40 ppm.

N⁶-(4 - Methoxytrityl) - 9 - (2 - O,O' - bio(S - (O-(4-methoxytrityl)-2-oxethylaufidyl)-2-thioethyl))phosphonyl-methoxyethyl)adenine (12).

A mixture of 296 mg (0.458 mmol) of 11 with 977 mg (2.29 nmol) of mono-O-(4-methoxytrityl) dithiodiethanol (7) in

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5 ml of pyridine is treated with 341 mg (1.15 mmol) of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole. After 3 days, the reaction mixture is diluted with dichloromethane and washed with saturated aqueous sodium hydrogen carbonate solution and then with water. The organic phase is dried over sodium sulfate, concentrated under reduced pressure, co-evaporated with toluene and chromatographed on a column of silica gel (eluent: methanol (0-5%) in dichloromethane) to give 330 mg (53%) of 12.

10 12: UV (EtOH): λ_{\max} 275 nm (ϵ 28200), λ_{\min} 253 nm (ϵ 18300)

MS (negative FAB, NBA) 1360 (M-H)-: 952 (M-MtrOCH₂CH₂SSCH₂CH₂)-.

¹H NMR (DMSO-d₆): 2.75 (t, 4H, 2 SCH₂CH₂OP, J = 6.3 Hz) 2.86 (t, 4H, 2 CH₂CH₂OMTr, J = 5.9 Hz); 3.19 (t, 4H, 2 CH₂OMTr, J = 6.0 Hz); 3.68 (s, 3H, CH₃O); 3.69 (s, 6H, 2 CH₃O); 3.83 (m, 4H, PCH₂ and CH₂CH₂); 4.05 (m, 4H, 2 CH₂OP); 4.28 (t, 2H, CH₂CH₂, J = 4.6 Hz); 6.87 - 7.45 (m, 42H, 3 Tr); 7.88 (s, 1H, H-8); 8.12 (s, 1H, H-2)ppm.

20 ³¹P NMR (DMSO-d₆): 22.09 ppm.

9-(2-(O,O'-Bis(S-(2-hydroxyethylsufidyl)-2-thioethyl)phosphonylmethoxyethyl)adenine (4).

The phosphotriester 12 (290 mg, 0.213 mmol) is treated with 15 ml of the acetic acid/water/methanol mixture (8:1:1) for 15 hours. The solvents are stripped off by evaporation under reduced pressure and the oil obtained is co-evaporated with toluene. Purification on a column of silica gel (eluent: methanol (0-8%) in dichloromethane) gives 116 mg (90%) of compound 4 after lyophilization in the water/dioxane mixture.

30 4: LTV (EtOH): λ_{\max} 260 nm (ϵ 14700); λ_{\min} 228 nm (ϵ 3600).

MS (positive FAB, GT): 545 (M+H).

35 ¹H NMR (DMSO-d₆): 2.80 (t, 4H, 2 SCH₂CH₂OP, J = 6.4 Hz); 2.91 (t, 4H, 2 SCH₂CH₂OH, J = 6.4 Hz); 3.61 (pseudo q, 4H, 2 CH₂OH, J = 6 Hz); 3.91 (t, 2H, CH₂CH₂, J = 5.1 Hz);

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3.95 (d, 2H, PCH_2 , $J = 8.2$ Hz); 4.15 (m, 4H, 2 CH_2OP); 4.32 (t, 2H, CH_2CH_2 , $J = 5.0$ Hz); 7.20 (bs, 2H, NH_2); 8.08 (s, 1H, H-8); 8.14 (s, 1H, H-2) ppm.

^{31}P NMR ($\text{DMSO}-d_6$): 22.24 ppm.

5 EXAMPLE 5:

EVALUATION OF THE ANTI-HIV I ACTIVITY ON CEM CELLS AND MT-4 CELLS

HIV = Human immunodeficiency virus

MT-4 = Human leukemia T cell

10 CEM = Human lymphoblastoid T cell

HIV-1 replication (LAI isolate) in CEM cells is measured by assaying the reverse transcriptase (RTase) in the culture supernatant after infection for 5 days. This activity reflects the presence of the virus released by the cells.

15 After adsorption of the virus, the test compounds are added, at various concentrations, to the culture medium.

Antiviral activity is expressed as the lowest concentration of compound which reduces the production of RTase by at least 50% (ED_{50}).

20 The toxic effect on non-infected CEMs is assessed by a calorimetric reaction based on the capacity of living cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into formazan after incubation for 5 days in the presence of various concentrations of the compounds. The results are expressed as the lowest concentration of compound which results in at least 50% inhibition of the formation of formazan (CD_{50}).

The compounds used as examples in this invention have the following anti-HIV activities:

30 Compound 1:

ED_{50}	-	CEM-TK-,	$4 \cdot 10^{-6}$ M	(CD_{50} $7 \cdot 10^{-5}$ M)
		CEM-SS,	$5 \cdot 10^{-6}$ M	(CD_{50} $9 \cdot 10^{-5}$ M)
		MT4 ,	$2 \cdot 10^{-6}$	(CD_{50} $9 \cdot 10^{-5}$ M)

Compound 2:

35

ED	-	CEM-TK-,	$8 \cdot 10^{-6}$ M	(CD_{50} $8 \cdot 10^{-5}$ M)
		CEM-SS,	$6 \cdot 10^{-5}$ M	(CD_{50} 10^{-4} M)

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Compound 3:

ED ₅₀ -	CEM-TK ⁺ ,	7.10 ⁻⁶ M	(CD ₅₀ 8.10 ⁻⁵ M)
	CEM-SS,	7.10 ⁻¹⁰ M	(CD ₅₀ 8.10 ⁻⁵ M)
	MT4	10 ⁻⁹ M	(CD ₅₀ 8.10 ⁻⁵ M)

5 Compound 4:

ED ₅₀ -	CEM-TK ⁺ ,	8.10 ⁻⁸ M	(CD ₅₀ 4.10 ⁻⁵ M)
	CEM-SS,	3.10 ⁻⁶ M	(CD ₅₀ > 10 ⁻⁴ M)
	MT4,	8.10 ⁻⁷ M	(CD ₅₀ 2.10 ⁻⁵ M)

This set of data shows that there has indeed been
 10 intracellular release of the nucleoside monophosphate.

EXAMPLE 6

O,O'-Bis(S-acetyl-2-thioethyl)-N,N-diisopropylphosphoramidite

To a stirred solution of N,N-diisopropylphosphorodichloridate (4.04 g, 20 mmol) in tetrahydrofuran (150 ml) at -
 15 78°C was added dropwise over 45 minutes a solution of S-acetylthioethanol (4.81 g, 40 mmol) and triethylamine (5.53, 40 mmol) in tetrahydrofuran (100 ml). The resulting reaction mixture was stirred for 2 hours at ambient temperature then filtered. The filtrate was concentrated under vacuum and the
 20 residue was diluted with cyclohexane and filtered. The filtrate was concentrated to a residue under vacuum, Diluted with cyclohexane, filtered and concentrate again. The final residue was chromatographed on a silica gel column. The column was eluted with a gradient of ethyl acetate in cyclohexane
 25 (0→20%) containing 5% triethylamine to obtain the title compound, O,O'-bis(S-acetyl-2-thioethyl)-N,N-diisopropylphosphoramidite (5.3 g, 72%).

Mass Spec (FAB positive, GT): 370 (M+H)⁺, 103 [CH₃C(O)SCH₂CH₂]⁺.

30 NMR ¹H (DMSO-d₆): 3.70-3.47 (m, 6H, 2 CH₂OP, 2 CH(CH₃)₂); 3.04 (t, 4H, 2SCH₂CH₂, J=6.4 Hz); 2.32 (s, 6H, 2CH₃COS); 1.10 (d, 12H, 2 CH(CH₃)₂), J = 6.8 Hz) ppm. NMR ³¹P (DMSO-d₆): 147.9 ppm(q).

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EXAMPLE 7

General procedure for O-(2',3'-dideoxynucleosid-5'-yl)-O'-O''-bis(S-acetyl-2-thioethyl)phosphates

To a solution of a 2',3'-dideoxynucleoside [AZT (0.1 g, 0.37 mmol); ddA (0.05 g, 0.5 mmol); ddI (0.12 g, 0.5 mmol); or ddT (0.11 g, 0.5 mmol)] and O,O'-bis(S-acetyl-2-thioethyl)-N,N'-diisopropylphosphoramidite (1.2 eq.) in a mixture of tetrahydrofuran/dimethylformamide (1:1, v/v, 5 ml per mmol) was added sublimed tetrazole (3.0 eq). After 30 min of stirring at ambient temperature the reaction mixture was cooled to -40 °C and a suspension of 3-chloroperbenzoic acid (1.3 eq) in dichloromethane (2 ml per mmol) was added. After stirring for one hour at ambient temperature the excess peracid was reduced with an aqueous solution of 10% sodium thiosulfate. The crude residue was diluted with dichloromethane and extracted with a saturated aqueous solution of sodium bicarbonate. The organic phase was wash with water, dried over sodium sulfate, filtered and evaporated under vacuum. The residue was chromatographed on a silica gel column eluted with a step gradient of methanol in dichloromethane to give the title bis(SATE)phosphotriesters as pure products.

EXAMPLE 8

O-(2',3'-Dideoxy-3'-azidothymidin-5'-yl)-O,O'-bis(S-acetyl-2-thioethyl)phosphate [Bis(SATE)AZTMP]

Prepared as per the above general procedure to give 0.11 g (53%) of the title compound.

UV (EtOH): λ_{\max} 264 nm (ϵ 9800), λ_{\min} 246 nm (ϵ 6500).

Mass Spec. (FAB positive, GT): 552 (M+H)⁺, (FAB negative, GT): 550 (M-H)⁻.

NMR ¹H (DMSO-d₆): 11.36 (s, 1H, NH-3); 7.46 (d, 1H, H-6, J_{H-6, CH-5} = 0.7 Hz); 6.13 (t, 1H, H-1', J_{H1',2'} = 6.7 Hz) 4.46 (m, 1H, H-3'); 4.20 (m, 2H, H-5',5''); 4.03 (m, 5H, H-4', CH₂-CH₂-O); 3.12 (t, 4H, S-C-H_{2a}-CH_{2b}, J_{Ha,Hb} = 6.3 Hz); 2.42 (m, 8H, H-2',2'', CH₃-CO); 1.78 (s, 3H, CH₃-5) ppm.

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EXAMPLE 9

O-(2',3'-Dideoxyadenosin-5'-yl)-O,O'-bis(S-acetyl-2-thioethyl)-phosphate [Bis(SATE)ddAMP]

Prepared as per the above general procedure to give
5 0.65 g (50%) of the title compound.

UV (EtOH): λ_{\max} 260 nm (ϵ 12000), 229 nm (ϵ 8600), λ_{\min} 240 nm (ϵ 7200), 223 nm (ϵ 7900).

Mass Spec. (FAB positive, GT): 520 (M+H)⁺, 136 (BH₂)⁺; (FAB negative, GT): 416 (M-CH₃C(O)SCH₂CH₂)⁻, 134 (B)⁻.

10 NMR ¹H (DMSO-d₆): 8.25 & 8.13 (2s, 1H & 1H, H-2 & H-8); 7.24 (s, 2H, NH₂); 6.24 (t, 1H, J = 5.4 Hz, H-1'); 4.28 (m, 1H, H-4'); 4.18-4.03 (m, 2H, H-5' & H-5''); 3.96 (q, 4H, 2 S-CH₂-CH₂-O); 3.06 (t, 4H, J = 6.3 Hz, 2 S-CH₂-CH₂-O); 2.48 (m, 2H, H-2' & H-2''); 2.32 & 2.31 (2s, 3H & 3H, 2 CH₃) ppm.

15 NMR ³¹P (DMSO-d₆) 0.78 ppm.

EXAMPLE 10

O-(2',3'-Dideoxyinosin-5'-yl)-O,O'-bis(S-acetyl-2-thioethyl)-phosphate [Bis(SATE)ddIMP]

Prepared as per the above general procedure to give
20 0.21 g (81%) of the title compound.

UV (EtOH) λ_{\max} 242 nm (ϵ 14700), 235 nm (ϵ 14900), shoulder 266 nm (ϵ 5800) & 248 nm (ϵ 13400).

Mass Spec. (FAB positive, GT): 521 (M+H)⁺, 137 (BH₂)⁺, 103 (CH₃C(O)SCH₂CH₂)⁺; (FAB negative, GT): 519 (M-H)⁻, 135 (B)⁻.

25 NMR ¹H (DMSO-d₆): 12.36 (s, 1H, NH-1); 8.21 (s, 1H, H-2); 8.04 (s, 1H, H-8); 6.22 (m, 1H, H-1'); 4.28 (m, 1H, H-4'); 4.20-4.02 (m, 2H, H-5' & H-5''); 3.97 (m, 4H, 2 S-CH₂-CH₂-OP); 3.07 (t, 4H, J = 6.4 Hz, 2 S-CH₂-CH₂); 2.49-2.42 (m, 2H, H-2' & H-2''); 2.33 (s, 3H, CH₃COS), 2.32 (s, 3H, CH₃COS),
30 2.15-2.02 (m, 2H, H-3' & H-3'') ppm.

NMR ³¹P (DMSO-d₆) 0.77 (m) ppm.

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EXAMPLE 11

O-(2',3'-Dideoxythymidin-5'-yl)-O,O'-bis(S-acetyl-2-thioethyl)-phosphate [Bis(SATE)ddTMP]

Prepared as per the above general procedure to give
5 0.23 g (91%) of the title compound.

UV (EtOH) λ_{\max} 266 nm (ϵ 8800), λ_{\min} 246 nm (ϵ 5400).

NMR ^1H (DMSO- d_6): 11.29 (s, 1H, NH-3); 7.47 (d, 1H, H-6; J = 1.0 Hz); 6.01 (m, 1H, H-1'), 4.20-4.11 (m, 3H, H-4', H-5', 5''); 4.04 (m, 4H, 2 $\text{CH}_2\text{-CH}_2\text{-OP}$); 3.11 (t, 4H, S-C- H_{2a} - CH_{2b} ,
10 J = 6.3 Hz); 2.34 (s, 3H, $\text{CH}_3\text{-COS}$); 2.33 (s, 3H, $\text{CH}_3\text{-COS}$); 2.33-2.25 (m, 1H, H-2''); 2.00-1.90 (m, 3H, H-2'', 3', 3''); 1.78 (d, 3H, $\text{CH}_3\text{-5}$, J = 0.6 Hz) ppm.

NMR ^{31}P (DMSO- d_6) 0.56 ppm.

EXAMPLE 12

15 N⁶-(4-Methoxytrityl)-9-(2-(O,O'-bis(S-acetyl-2-thioethyl)-phosphonylmethoxyethyl)adenine [Bis(SATE)PMEA-MTr]

To a solution of N⁶-(4-methoxytrityl)-9-(2-phosphonylmethoxyethyl)adenine (compound 11) as a mixture of triethylammonium salts (0.25:0.75, 0.3 g, 0.43 mmol), 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (0.41 g, 1.38 mmol)
20 in anhydrous pyridine (6 ml) was added S-acetylthioethanol (0.33 g, 2.77 mmol). The reaction mixture was stirred overnight at ambient temperature and then neutralized with an aqueous triethylammonium bicarbonate (1M, pH 7.5, 4 ml).
25 Chloroform and water were added, the organic phase was decanted, dried over sodium sulfate, filtered and evaporated under vacuum. The residue was chromatographed on a silica gel column eluted with a gradient methanol in dichloromethane (0-2%) to give the title compound, bis(SATE)PMEA-MTr (0.15 g,
30 50%), as an oil.

Mass Spec. (FAB positive, GT): 750 (M+H)⁺.

NMR ^1H (DMSO- d_6): 8.15 & 7.90 (2s, 1H & 1H, H-2 & H-8); 7.31-6.81 (m, 15H, trityl & NH); 4.32 (t, 2H, J = 4.7 Hz, CH_2N); 3.99-3.84 (m, 8H, 2 S- $\text{CH}_2\text{-CH}_2\text{-O}$, $\text{CH}_2\text{-P}$, $\text{CH}_2\text{-CH}_2\text{-N}$); 3.70
35 (s, 3H, OCH_3); 3.01 (t, 4H, J = 6.4 Hz, S- $\text{CH}_2\text{-CH}_2\text{-O}$); 2.30 (s, 6H, 2 CH_3) ppm.

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NMR ^{31}P (DMSO- d_6) 22.51 ppm.

EXAMPLE 13

9-(2-(O,O'-Bis(S-acetyl-2-thioethyl)phosphonylmethoxyethyl)-adenine [Bis(SATE)PMEA]

5 A solution of bis(SATE)PMEA^{MT} (0.21 g, 0.28 mmol) in acetic acid:water:methanol (8:1:1, v/v/v, 22 ml) was stirred overnight at ambient temperature. The reaction mixture was evaporated and the residue co-evaporated with 100% ethanol and dichloromethane. The residue was chromatographed on a silica
10 gel column to give pure bis(SATE)PMEA (0.079 g, 59%). m.p. 66 °C (crystallized from toluene).

UV (EtOH) λ_{max} 260 nm (ϵ 14200), 230 nm (ϵ 10400), λ_{min} 240 nm (ϵ 9200), 223 nm (ϵ 9800).

Mass Spec. (FAB positive, GT): 570 (M+G+H)⁺, 478
15 (M+H)⁺; (FAB negative, GT): 374 (M-CH₃C(O)SCH₂CH₂)⁻.

NMR ^1H (DMSO- d_6): 8.12 & 8.06 (2s, 1H & 1H, H-2 & H-8); 7.17 (s, 2H, NH₂); 4.31 (t, 2H, J = 5.0 Hz, CH₂N); 4.00-3.86 (m, 8H, 2 S-CH₂-CH₂-O, CH₂-P, CH₂-CH₂-N); 3.03 (t, 4H, J = 6.4 Hz, 2 S-CH₂-CH₂-O); 2.33 (s, 6H, 2 CH₃) ppm.

20 NMR ^{31}P (DMSO- d_6) 22.53 ppm.

EXAMPLE 14

EVALUATION OF THE ANTI-HIV 1 ACTIVITY ON CEM CELLS AND MT-4 CELLS OF BIS(SATE)PHOSPHOTRIESTERS OF AZT, ddA, ddI, ddT AND PMEA

25 The compounds were tested as described in Example 5 above.

-AZT

30	ED ₅₀	-	CEM-TK	> 10 ⁻⁴ M	(CD ₅₀ > 10 ⁻⁴ M)
			CEM-SS	4.8 10 ⁻⁹ M	(CD ₅₀ > 10 ⁻⁴ M)
				± 2.4 10 ⁻⁹	
			MT-4	1.8 10 ⁻⁸ M	(CD ₅₀ > 10 ⁻⁴ M)
				± 0.6 10 ⁻⁸	

-Bis(SATE)AZTMP

35	ED ₅₀	-	CEM-TK	3.9 10 ⁻⁸ M	(CD ₅₀ ND)
			CEM-SS	2.2 10 ⁻⁸ M	(CD ₅₀ ND)

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		MT-4	$7.8 \cdot 10^{-8} \text{ M}$	$(\text{CD}_{50} \cdot 7.6 \cdot 10^{-5} \text{ M})$
	-ddA			
	ED ₅₀	CEM-TK	$1.1 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
		CEM-SS	$5.4 \cdot 10^{-7} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
5			$\pm 1.1 \cdot 10^{-7}$	
		MT-4	10^{-5} M	$(\text{CD}_{50} > 10^{-4} \text{ M})$
	-Bis(SATE)ddAMP			
	ED ₅₀	CEM-TK	$7.7 \cdot 10^{-10} \text{ M}$	$(\text{CD}_{50} > 10^{-5} \text{ M})$
		CEM-SS	$5.6 \cdot 10^{-10} \text{ M}$	$(\text{CD}_{50} \cdot 2.4 \cdot 10^{-5} \text{ M})$
10			$\pm 3.4 \cdot 10^{-10}$	$\pm 0.1 \cdot 10^{-5}$
		MT-4	$1.1 \cdot 10^{-8} \text{ M}$	$(\text{CD}_{50} \cdot 1.6 \cdot 10^{-5} \text{ M})$
			$\pm 0.8 \cdot 10^{-8}$	$\pm 0.9 \cdot 10^{-5}$
	-ddI			
	ED ₅₀	CEM-TK	$9.5 \cdot 10^{-7} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
15		CEM-SS	$4.3 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
			$\pm 2.0 \cdot 10^{-6}$	
		MT-4	$1.1 \cdot 10^{-5} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
			$\pm 0.2 \cdot 10^{-5}$	
	-Bis(SATE)ddIMP			
20	ED ₅₀	CEM-TK	$3.0 \cdot 10^{-7} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
		CEM-SS	$1.2 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
			$\pm 0.6 \cdot 10^{-5}$	
		MT-4	$3.4 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
			$\pm 1.1 \cdot 10^{-6}$	
25	-ddT			
	ED ₅₀	CEM-TK	$> 10^{-4} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
		CEM-SS	$4.0 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
		MT-4	ND	$(\text{CD}_{50} \text{ ND})$
	-Bis(SATE)ddTMP			
30	ED ₅₀	CEM-TK	$5 \cdot 10^{-7} \text{ M}$	$(\text{CD}_{50} > 10^{-4} \text{ M})$
		CEM-SS	$1.7 \cdot 10^{-6} \text{ M}$	$(\text{CD}_{50} \cdot 8.5 \cdot 10^{-5} \text{ M})$
		MT-4	ND	$(\text{CD}_{50} \text{ ND})$

In the same manner as was seen for the activities exhibited in Example 5, the anti HIV activity of the above listed bis(SATE) derivatives show increases of up to 1/3 log

- 25 -

units compared to their parent nucleosides (compare AZTMP and ddTMP to the parent nucleosides AZT and ddT, respectively). This increase in activity shows that there was intercellular release of the nucleoside monophosphate.

5 For Examples 15 and 16 below, ^1H NMR were recorded using a Bruker AC 250 or a Bruker AC 400 spectrometer at ambient temperature in CDCl_3 . Chemical shifts are given in δ -values referenced to the residual solvent peak (7.26 ppm). Deuterium exchange, decoupling and COSY experiments were
10 performed in order to confirm proton assignments. ^{31}P NMR spectra were recorded at ambient temperature on a Bruker AC 250 spectrometer at 101.2 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4 . ^{13}C NMR spectra were measured on a Bruker AC 400 spectrometer at 100.6 MHz with
15 proton decoupling using CDCl_3 (77.00 ppm) as internal standard. Coupling constants, J , are reported in hertz. FAB mass spectra were reported in the positive-ion or negative-ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system using thioglycerol/glycerol (1:1, v/v, G-T) as
20 matrix. Xe atoms were used for the gun at 3 kV with a total discharge current of 20 mA. UV spectra were recorded on an Uvikon 810 (Kontron) spectrometer in ethanol (95%).

TLC was performed on precoated aluminum sheets of silica gel 60 F_{254} (Merck), visualization of products being
25 accomplished by UV absorbance followed by charring with 5% ethanolic sulfuric acid with heating; phosphorus-containing compounds were detected by spraying with Hanes molybdate reagent (Hanes et. al., *Nature*, 154, 1107-1112, 1949). Column chromatography was carried out on silica gel 60 (Merck).

30 High-performance liquid chromatography (HPLC) studies were carried out on a Waters Assoc. unit equipped with a model 616 pump system, a model 600S system controller, a model 996 photodiode array detector and a Millennium data workstation. The column was a reverse phase analytical column (Macherey-
35 Nagel, C_{18} , 150 x 4.6 mm, 5 μm) protected by a prefilter and a precolumn (Nucleosil, C_{18} , 5 μm). The compound to be analyzed

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was eluted using a linear gradient of 0% to 80% acetonitrile in 50 mM triethylammonium acetate buffer (pH 7) programmed over a 40 min period with a flow rate of 1 ml/min and detection at 260 nm.

5 Evaporation of solvents was carried out on a rotary evaporator at 40°C or lower under reduced pressure. Dichloromethane and 2-mercaptoethanol were distilled over calcium hydride and acetonitrile was dried over phosphorus pentoxide. Anhydrous *N,N*-dimethylformamide (Fluka) was used
10 as supplied. All solvents used in reactions involving trivalent phosphorus compounds were degased by an argon stream before use. All reactions were carried out under rigorous anhydrous conditions under an argon atmosphere.

Tris(pyrrolidino)phosphine was prepared as described
15 by Wiesler et al. (Wiesler et al., *Methods in molecular biology: Protocols for nucleotides and analogs* (S. Agrawal Ed.), Humana Press Inc., Totowa, NJ., Vol. 20, 1991-206, 1993. d4T and ddA were supplied by Sigma (D1413) and Fluka (36769) respectively and were dried over P₂O₅ under reduced pressure at
20 RT prior to use. Sublimed 1-*H*-Tetrazole was purchased from Sigma and used as supplied. Elemental sulfur and trimethylacetic anhydride were purchased from Aldrich, *tert*-butyl hydroperoxide (3M in toluene) from Fluka.

EXAMPLE 15

25 2-Mercaptoethyl-1-pivaloate

The title compound was prepared by adapting a method described by Miles et al., *J. Chem. Soc.*, 817-826, 1952. Trimethylacetic anhydride (5.8 ml, 28.5 mmol) was added dropwise to a mixture of 2-mercaptoethanol (2.0 ml, 28.5 mmol)
30 and sulfuric acid in acetic acid (0.09 ml, 10%, v/v) at 0°C. The solution was heated for 1h at 60-65°C and stirred during 3h at room temperature. After dilution with diethylether (40 ml), the reaction mixture was neutralized with saturated NaHCO₃ solution (10 ml), the organic layer was separated, washed with
35 water (3 x 10 ml), dried over sodium sulfate, filtered and evaporated. The residue was distilled under reduced pressure

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(bp₁₁ = 63-64°C) to yield 2.2 g (13.7 mmol, 48%) of **4** as a colorless oil.

R_f 0.64 (ethyl acetate/toluene 2:8);

¹H NMR (CDCl₃) δ 1.17 (s, 9H, tBu), 1.43 (t, 1H, SH, J = 8.5 Hz), 2.66-2.75 (m, 2H, SCH₂CH₂), 4.14 (t, 2H, CH₂CH₂O).

EXAMPLE 16

General procedure for the preparation of the phosphorodithiolates *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate and *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxy-2',3'-didehydrothymidin-5'-yl phosphorodithiolate

The appropriate nucleoside (0.5 mmol) was dissolved either in a mixture of DMF and dichloromethane (d4T, 1:3, 6 ml) or in DMF (ddA, 6 ml) by warming the solution at 50°C. After cooling to room temperature, the solution was stirred for 2 h over 3Å molecular sieve (0.5 g). Tris(pyrrolidino)phosphine **1**² (120 mg, 0.55 mmol) was added, followed by 1*H*-tetrazole in seven aliquots (7 x 50 µl of 0.5M tetrazole in acetonitrile, 0.175 mmol) at 3 min intervals. After stirring at room temperature for 15 min, 1*H*-tetrazole (5.2 ml 0.5M tetrazole in acetonitrile, 2.60 mmol) was added, immediately followed by the addition of the thiol **4** (243 mg, 1.5 mmol). The reaction mixture was stirred at room temperature for 45 min and then cooled to -40°C. *tert*-Butyl-hydroperoxide (360 µl, 3M in toluene) was added and the reaction mixture allowed to warm to room temperature over 45 min. The reaction mixture was concentrated under reduced pressure to approximately 2 ml and diluted with dichloromethane (10 ml). The excess of oxidant was destroyed by the addition of Na₂S₂O₃ (10%, 5 ml). The organic phase was separated and the aqueous phase extracted twice with dichloromethane (2 x 10 ml). The combined organic layers were successively washed with brine (10 ml) and water (10 ml). The organic layer was dried with sodium sulfate, filtered and concentrated to dryness under reduced pressure. Column chromatography of the residue on silica gel afforded the title compounds.

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EXAMPLE 16A

S,S'-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate

85 mg, 0.14 mmol, 28% after chromatography [eluent, stepwise gradient of methanol (3→5%) in dichloromethane].

R_f 0.30 (methanol/dichloromethane 1:9);

^1H NMR (CDCl_3) δ 1.20 (s, 18H, tBu), 2.19-2.28 (m, 2H, 3'-H, 3''-H), 2.48-2.68 (m, 2H, 2'-H, 2''-H), 3.07-3.20 (m, 4H, SCH_2CH_2), 4.29 (t, 4H, $\text{CH}_2\text{CH}_2\text{O}$, $J = 6.4$ Hz), 4.30-4.48 (m, 3H, 4'-H, 5'-H, 5''-H), 5.3 (bs, 2H, NH_2), 6.31 (q, 1H, 1'-H, $J = 3.9, 6.5$ Hz), 8.07, 8.35 (2s, 2H, 2-H, 8-H);

^{13}C NMR (CDCl_3) δ 26.03 (C-3'), 27.05 ($\text{C}(\text{CH}_3)_3$), 30.42, 30.45 (2d, SCH_2CH_2 , $J_{\text{p-c}}$ 14 Hz), 32.16 (C-2'), 38.72 ($\text{C}(\text{CH}_3)_3$), 62.56, 62.58 (2d, OCH_2CH_2 , $J_{\text{p-c}}$ 5 Hz), 67.93 (d, C-5', $J_{\text{p-c}}$ 8 Hz), 79.20 (d, C-4', $J_{\text{p-c}}$ 8 Hz), 85.51 (C-1'), 120.02 (C-5), 138.71 (C-8), 149.23 (C-4), 152.82 (C-2), 155.48 (C-6), 177.95 (C=O);

^{31}P NMR (CDCl_3) δ 57.09 (s);

FAB MS (>0 , G=T) $m-e$ 604 [$\text{M} + \text{H}$] $^+$;

FAB MS (<0 , G-T) $m-e$ 602 [$\text{M} - \text{H}$]; 474 [OPS(SR)(OddA)]; 385 [OPO(SR) $_2$];

UV (ethanol 95) λ_{max} 259 nm (ϵ 14900);

HPLC t_R 29.5 min.

EXAMPLE 16B

S,S'-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxy-2',3'-didehydrothymidin-5'-yl phosphorodithiolate

98 mg, 0.17 mmol, 33% after column chromatography (eluent ethylacetate/dichloromethane 3:7);

R_f 0.28 (dichloromethane/ethyl acetate 4:6);

^1H NMR (CDCl_3) δ 1.19, 1.20 (2s, 18H, tBu), 1.94 (d, 3H, thymine- CH_3 , $J = 1.2$ Hz), 3.09-3.21 (m, 4H, SCH_2CH_2), 4.24-4.34 (m, 5H, $\text{CH}_2\text{CH}_2\text{O}$, 5'-H), 4.37-4.48 (m, 1H, 5''-H), 5.03-4.29 (m, 1H, 4'-H), 5.91-5.93 (m, 1H, 2'-H), 6.34-6.36 (m, 1H, 3'-H), 7.01-7.03 (m, 1H, 1'-H), 7.20 (d, 1H, 6-H, $J = 1.1$ Hz), 8.80 (bs, 1H, NH);

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^{13}C NMR (CDCl_3) δ 12.56 (thymine- CH_3), 27.08 ($\text{C}(\text{CH}_3)_3$), 30.51, 30.54 (2d, SCH_2CH_2 , $J_{\text{p-c}}$ 40 Hz), 38.76 ($\text{C}(\text{CH}_3)_3$), 62.59, 62.63 (2d, OCH_2CH_2 , $J_{\text{p-c}}$ 13 Hz), 67.15 (d, C-5', $J_{\text{p-c}}$ 8 Hz), 84.15 (d, C-4', $J_{\text{p-c}}$ 9 Hz), 89.58 (C-1'), 111.43 (C-5), 127.84 (C-2'), 132.86 (C-3'), 135.48 (C-6), 150.69 (C-2), 163.55 (C-4), 177.94 (C=O);

^{31}P NMR (CDCl_3) δ 57.29 (s);

FAB MS (>0 , G-T) $m-e$ 1185 [$2\text{M} + \text{H}$] $^+$;

FAB MS (<0 , G-T) $m-e$ 1183 [$2\text{M} - \text{H}$]; 591. [$\text{M}-\text{H}$]; 463
10 [OPS(SR)(Od4T)]; 385 [OPO(SR) $_2$];

UV (ethanol 95) λ_{max} 264 nm (ϵ 7250);

Anal. Calcd for $\text{C}_{24}\text{H}_{17}\text{N}_2\text{O}_9\text{PS}_2$: C, 48.63; H, 6.29; N, 4.73; S, 10.82; Found: C, 48.65; H, 6.38; N, 4.73; S, 10.97.

HPLC t_R 29.6 min.

15 EXAMPLE 17

Stability studies of isosteric S,S' -bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate and *O*-(2',3'-dideoxyadenosin-5'-yl)-*O*,*O'*-bis(*S*-acetyl-2-thioethyl) phosphate

Certain kinetic studies comparing the compound of
20 example 16A, i.e., S,S' -bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate, also designated as iso[Bis(SATE)ddA] and its isosteric compound of Example 9, i.e., *O*-(2',3'-dideoxyadenosin-5'-yl)-*O*,*O'*-bis(*S*-acetyl-2-thioethyl) phosphate, also designated as
25 [Bis(SATE)ddAMP] were effected. The kinetic were studies were performed in culture medium (RPMI containing 10% heat-inactivated fetal calf serum) in order to evaluate the stability of the pronucleotides in the extracellular medium used for antiviral evaluation in cell culture systems, and in
30 total cell extracts that mimic the behavior of the compounds inside cells. The "on-line ISPR cleaning" HPLC method of Lefebvre et al., J. Med. Chem., 38, 3941-3950, 1995, were used for the studies. The products resulting from the decomposition were characterized by co-injection with authentic samples
35 and/or by coupled HPLC/Mass Spectroscopy. The kinetics of transformation of the two isomeric pronucleotides strongly

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differed according to the medium. In culture medium the first iso-SATE group was cleaved in 38.5 hr whereas the first SATE group was cleaved in 165 hr. The second iso-SATE group was cleaved in 5.8 hr whereas the second SATE group was cleaved in 46 hr. In total cell extracts, the first iso-SATE group was cleaved in 5.3 hr whereas the SATE group was cleaved in 1 hr.

EXAMPLE 18

Decomposition pathway of *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate, i.e. iso[Bis(SATE)ddA], and *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxy-2',3'-didehydrothymidin-5'-yl phosphorodithiolate, i.e. iso[Bis(SATE)d4T]

The proposed decomposition pathway for the compounds of Example 16a and 16b, i.e., *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxyadenosin-5'-yl phosphorodithiolate and *S,S'*-bis(*O*-pivaloyl-2-oxyethyl) *O*-2',3'-dideoxy-2',3'-didehydrothymidin-5'-yl phosphorodithiolate, also designated as iso[Bis(SATE)ddA] and iso[Bis(SATE)d4T] is shown in Figure 9. This pathway was studied in conjunction with the kinetic studies of Example 17. While we do not wish to be bound by theory, based upon these results it is presently believed that the decomposition pathway for the iso-SATE pronucleotide involves:

(a) carboxyesterase-mediated cleavage of the ester function leading to (A);

(b) nucleophilic attack of the liberated hydroxyl function on the phosphorus atom, forming the five-covalent intermediate (B);

(c) conversion of the intermediate (B) into the 2-mercaptoethyl phosphorotriester (C);

(d) spontaneous elimination of episulfide, leading to the corresponding phosphorothiolate diester (D); and

(e) hydrolysis of the phosphorothiolate diester (D) into the corresponding 5'-monophosphate by a similar mechanism (a-b-c-d) or following the action of phosphodiesterases.

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Additionally, the hydrolysis of the iso-SATE pronucleotide may involve a direct nucleophilic attack on the phosphorus atom, leading directly to the phosphothiolate diester (D). Again while we do not wish to be bound by theory, we believe this might explain the faster decomposition of the iso-SATE pronucleotide compound to that of the SATE pronucleotide compound in culture medium.

EXAMPLE 19

Anti-HIV activity of mononucleoside S,S'-bis(O-pivaloyl-2-oxyethyl) phosphorodithiolates

The iso-SATE pronucleotides of Examples 16A and 16B were evaluated for their inhibitory effects on the replication of HIV-1 in CEM-SS and in thymidine-kinase deficient cell lines (CEM/TD). For comparison, the parent nucleosides ddA and d4T, and the corresponding bis(SATE) phosphotriesters bis(tBuSATE)ddAMP (the compound of Example 9) and bis(tBuSATE)d4TMP, were evaluated in the same experiments. The results are shown in Figure 10. In the two cell culture systems, the anti-HIV-1 activities of the tBu(iso)SATE pronucleotide were similar to those of their corresponding tBu(SATE) pronucleotides, both types of isomeric pronucleotides being more potent inhibitors than the parent compound ddA. The d4T derivative showed high inhibitory effects in thymidine-deficient (TK) CEM cells while, as expected, the d4T is weakly active in this cell line. This data clearly demonstrates that the isoSATE pronucleotides that were evaluated could act as efficient prodrugs forms of the 5'-monophosphates, circumventing the first activation step by cytosolic kinases.

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WHAT IS CLAIMED IS:

1. A compound corresponding to the general formula Ia:



in which:

R is a radical $-(\text{CH}_2)_n\text{-W-X}$, where X represents a radical $-\text{C(=Z)(Y)}$ or $-\text{S-U}$, and Z is O or S;

W is O or S;

Q is O or S;

Y and U represent an alkyl, aryl or saccharide radical which is optionally substituted, in particular with an OH, SH or NH group; and

n is equal to 1 to 4, preferably 1 or 2; and

Nu is a radical consisting of a residue of a biologically active compound or the dephosphorylated residue of a compound which is biologically active when it bears a phosphate or phosphonate group.

2. The compound of claim 1 wherein X represents $-\text{S-U}$ and U represents the radical $(\text{CH}_2)_{n^1}\text{-X}^1$ where X^1 represents H, OH, SH or NH_2 and n^1 is equal to 1 to 4.

3. The compound of claim 2 wherein R represents $-(\text{CH}_2)_2\text{-S-S-(CH}_2)_2\text{-OH}$.

4. The compound of claim 1 wherein X represents $-\text{C(=Z)Y}$ and Y represents CH_3 or tBu.

5. The compound of claim 4 wherein R represents $-(\text{CH}_2)_n\text{-S-C(=O)-CH}_3$ or $(\text{CH}_2)_n\text{-S-C(=O)-tBu}$ with $n = 1$ or 2 .

6. The compound of claim 1 wherein Nu represents a 5' residue of a natural nucleoside or of a derivative of a natural nucleoside, which is therapeutically active or for which the 5'-(O)-monophosphate or 5'-(C)-monophosphonate is therapeutically active.

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7. The compound of claim 6 wherein Nu represents a 5' residue of 2',3'-dideoxynucleoside or 2',3'-dideohydronucleoside.

8. The compound of claim 7 wherein Nu represents a 5' residue of dideoxyuridine, dideoxythymidine, dideoxycytidine, 3'-azido-2',3'-dideoxythymidine, or a derivative thereof which is substituted on the pyrimidine base or at 2' and 3' of the saccharide ring.

9. The compound of claim 1 wherein Nu represents a nucleoside analog residue such as a carbonucleoside, a phosphonucleoside, or an acyclonucleoside.

10. The compound of claim 9 wherein Nu is a radical of methoxyalkylpurine or methoxyalkylpyrimidine type, of formula $\text{CH}_2\text{-O-alkylpurine}$ or $\text{CH}_2\text{-O-alkylpyrimidine}$.

11. The compound of claim 10 wherein Nu is a 3-hydroxy-2-methoxypropylpurine or 3-hydroxy-2-methoxypropylpyrimidine radical of formula, of formula $\text{-CH}_2\text{-OCH(CH}_2\text{OH)-CH}_2\text{-purine}$ or $\text{-CH}_2\text{-OCH(CH}_2\text{OH)-CH}_2\text{-pyrimidine}$ or a 2-methoxyethylpurine or 2-methoxyethylpyrimidine radical, of formula $\text{-CH}_2\text{-O-C}_2\text{H}_4\text{-purine}$ or $\text{-CH}_2\text{-O-C}_2\text{H}_4\text{-pyrimidine}$.

12. A process for the preparation of a compound of claim 1 comprising the steps of appending protecting groups to said R groups and then removing said protecting groups to provide said compound.

13. The process of claim 13 wherein a compound of formula (II):



is reacted a compound of formula (III):



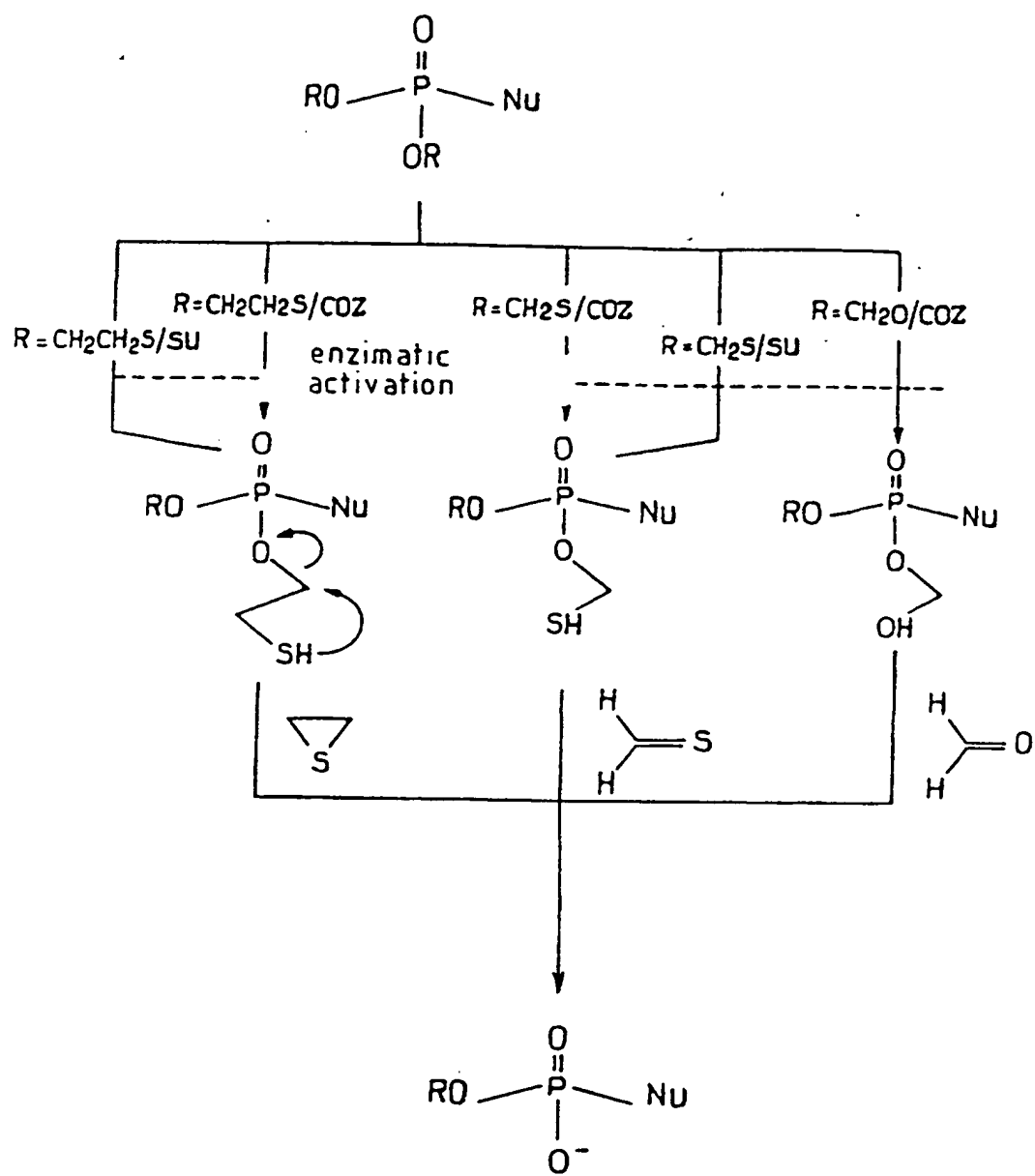
where X is protected, to provide said protected compound of formula (I); and

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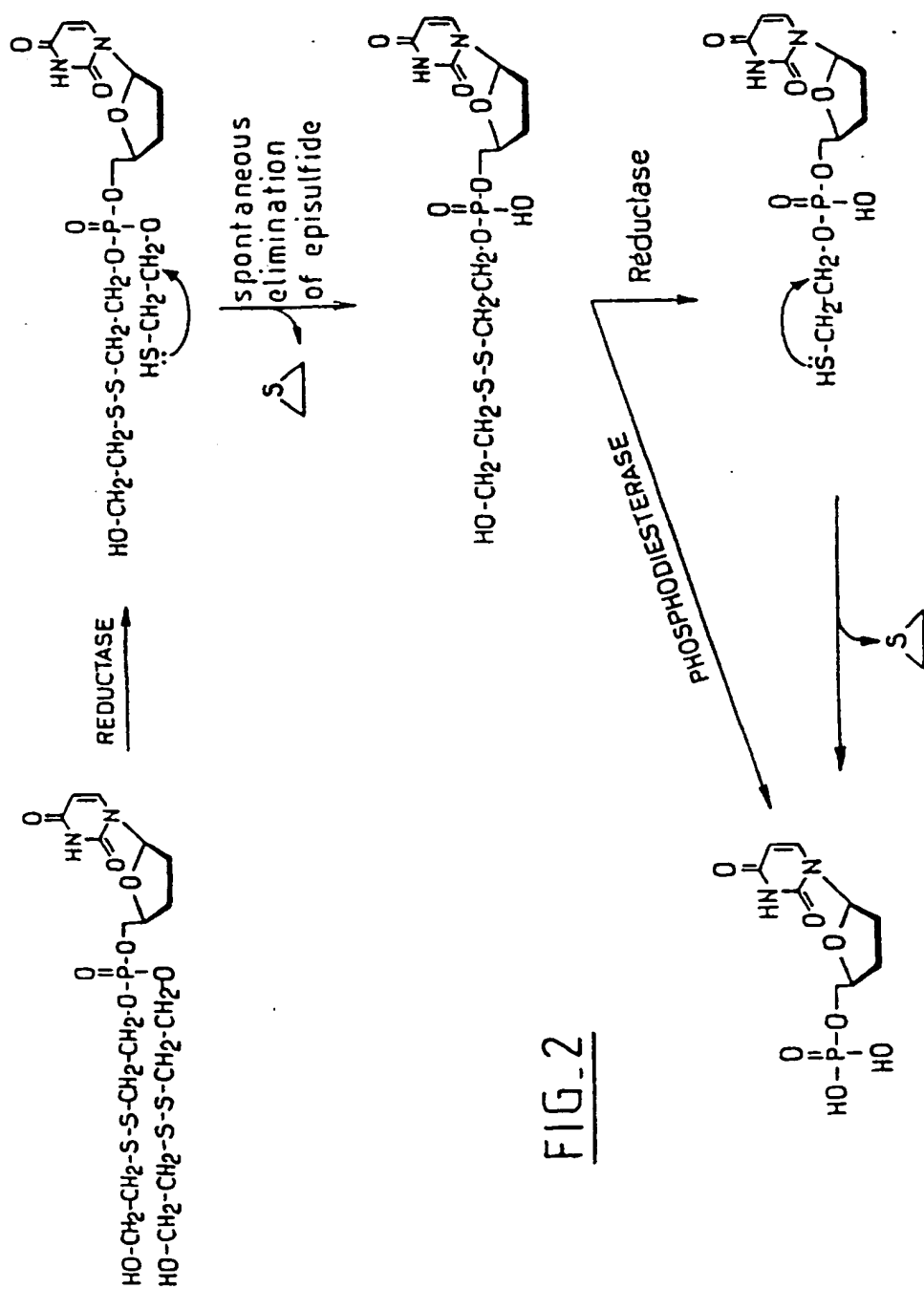
said protected compound of formula (I) is deprotected.

14. The process of claim 13 wherein the reaction between the compounds of formulas (II) and (II) takes place in the presence of a condensing agent in pyridine.

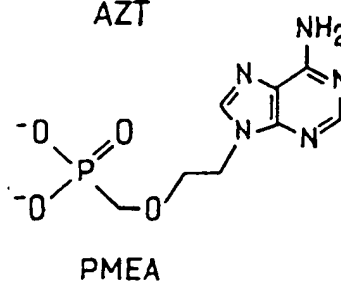
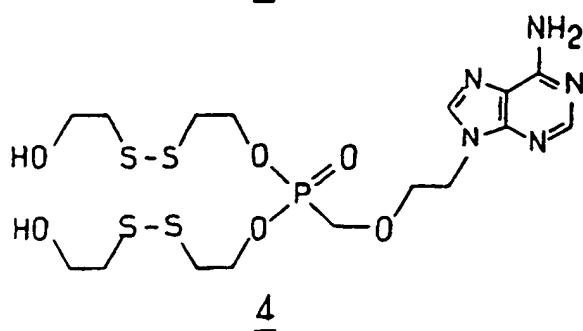
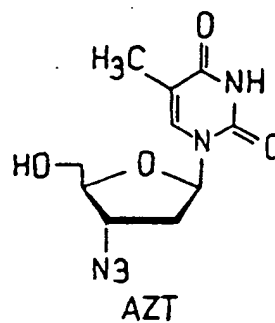
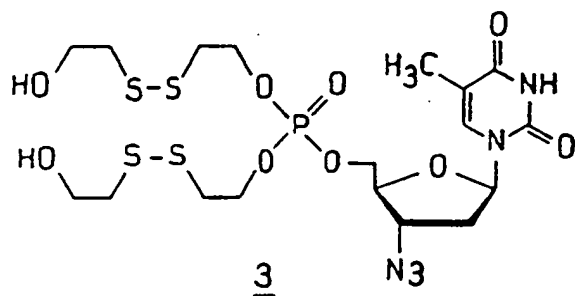
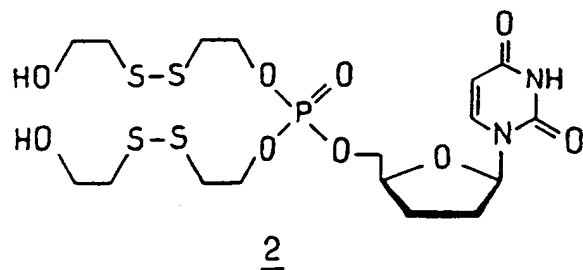
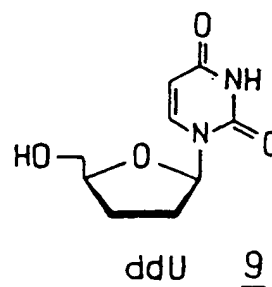
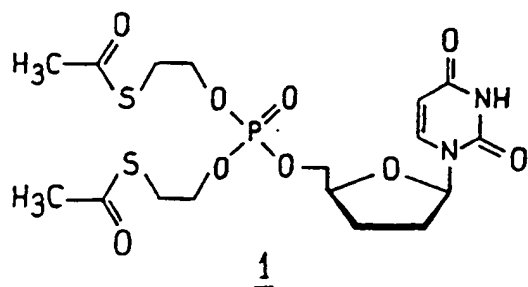
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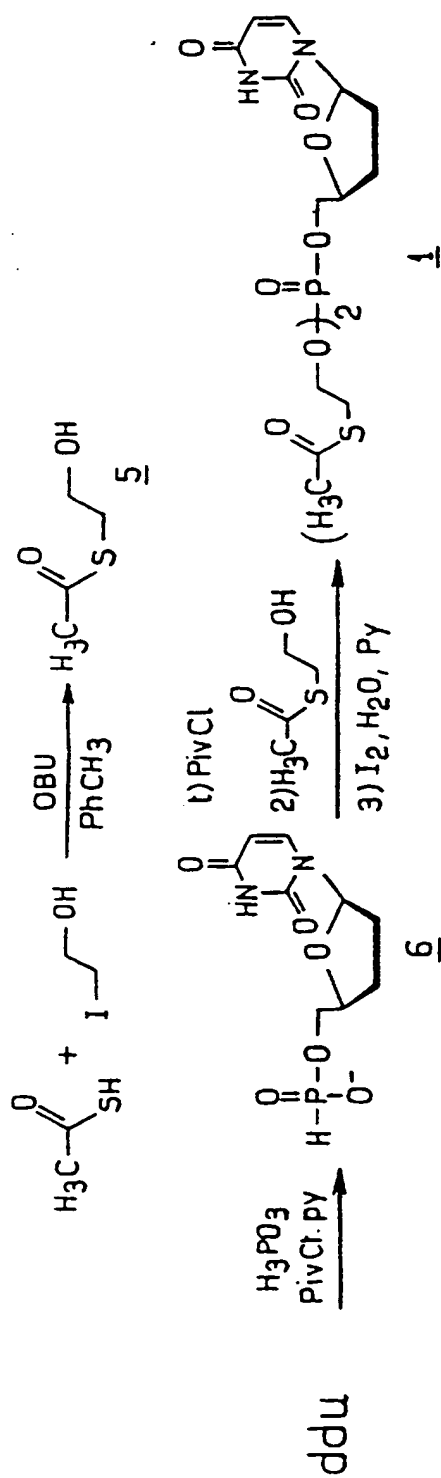
FIG. 1

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FIG. 2

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FIG. 3



Scheme 2

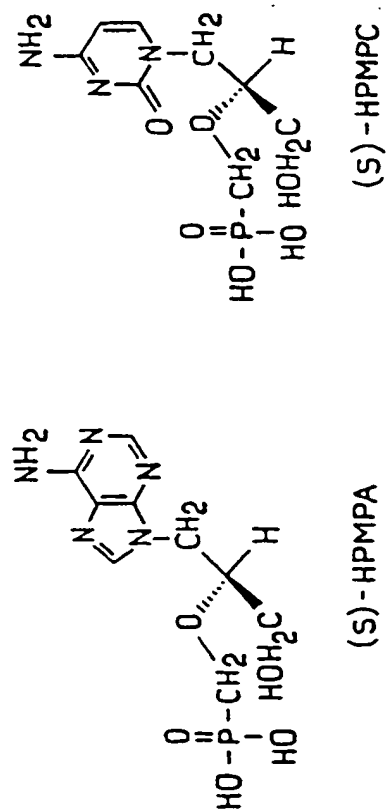


FIG. 4

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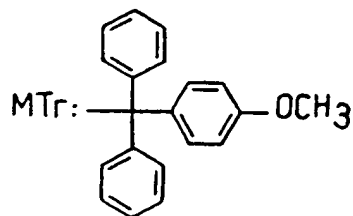
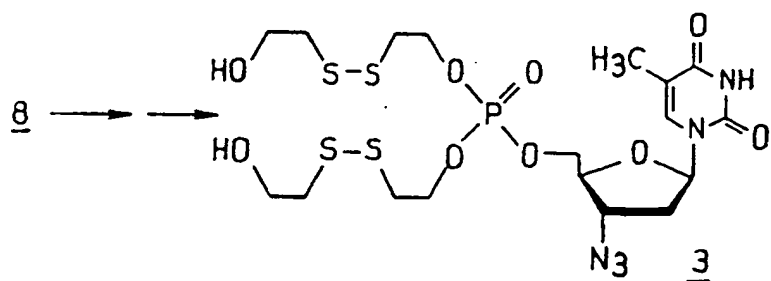
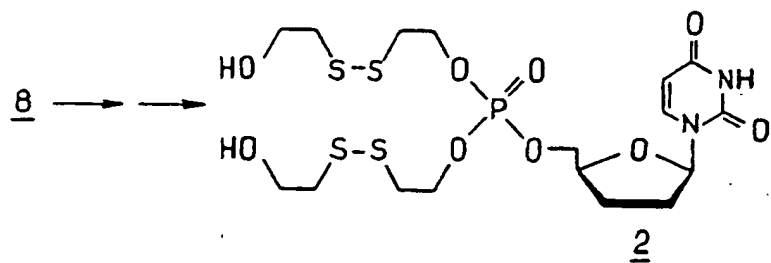
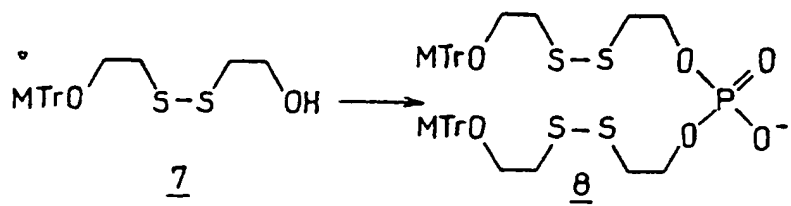
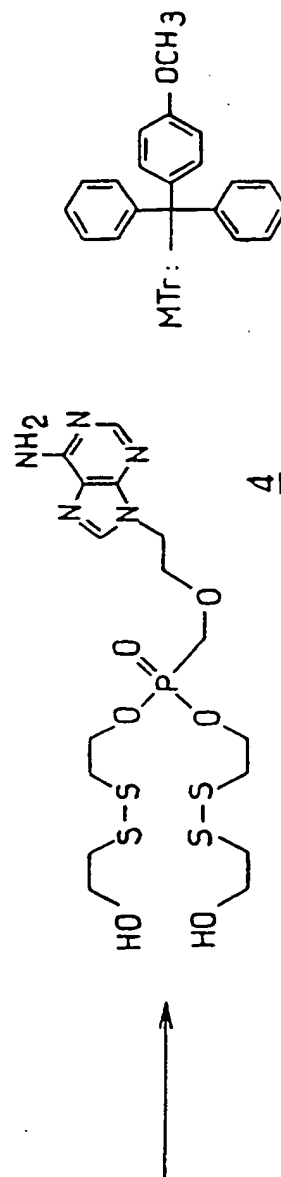
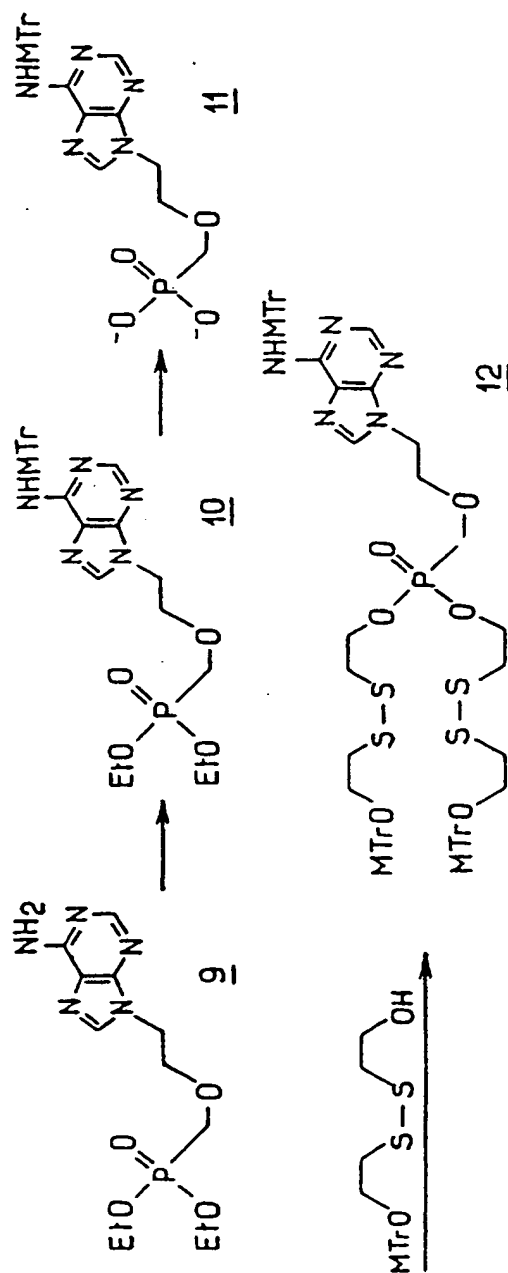


FIG. 5

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Scheme 3

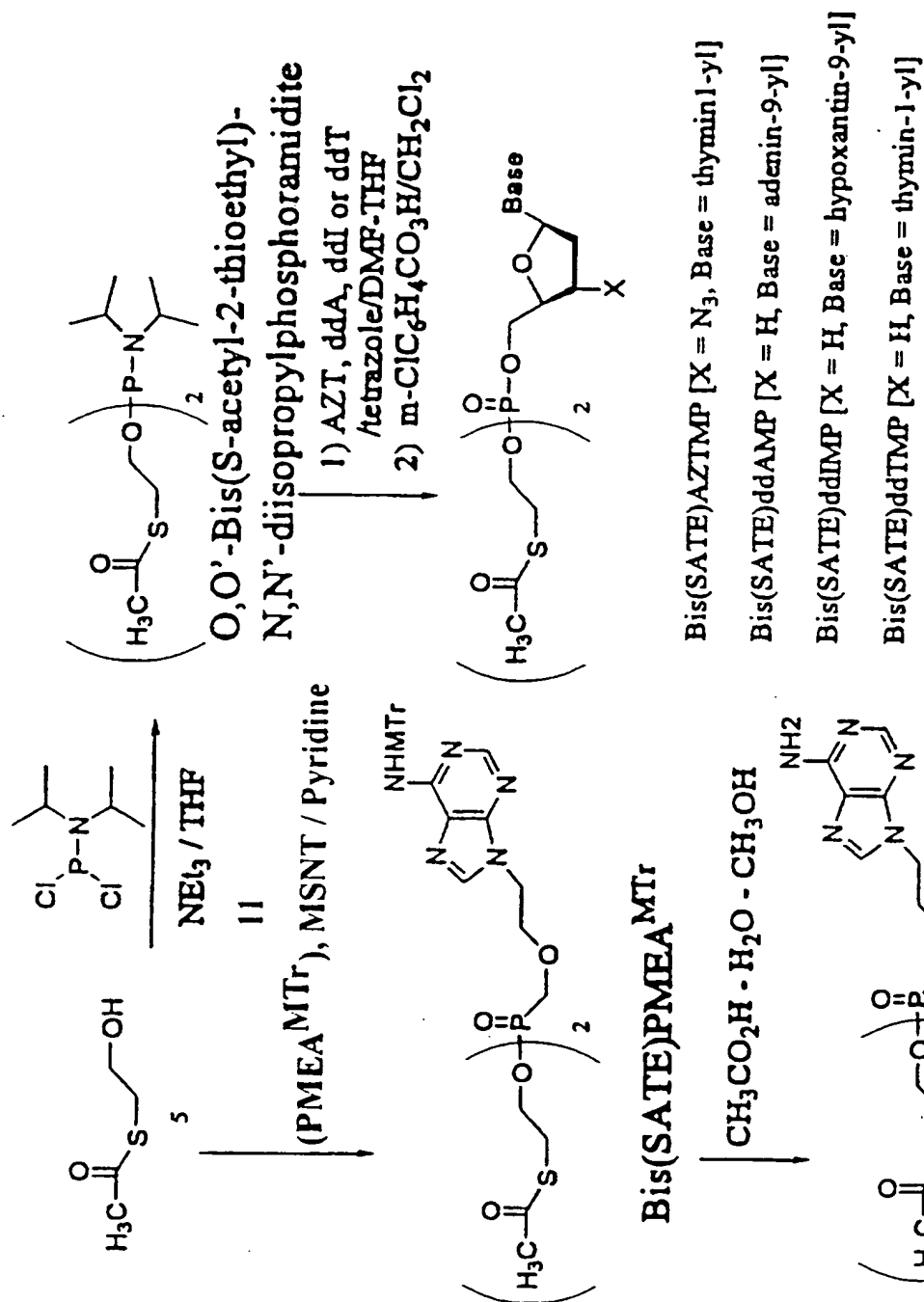


Figure 7

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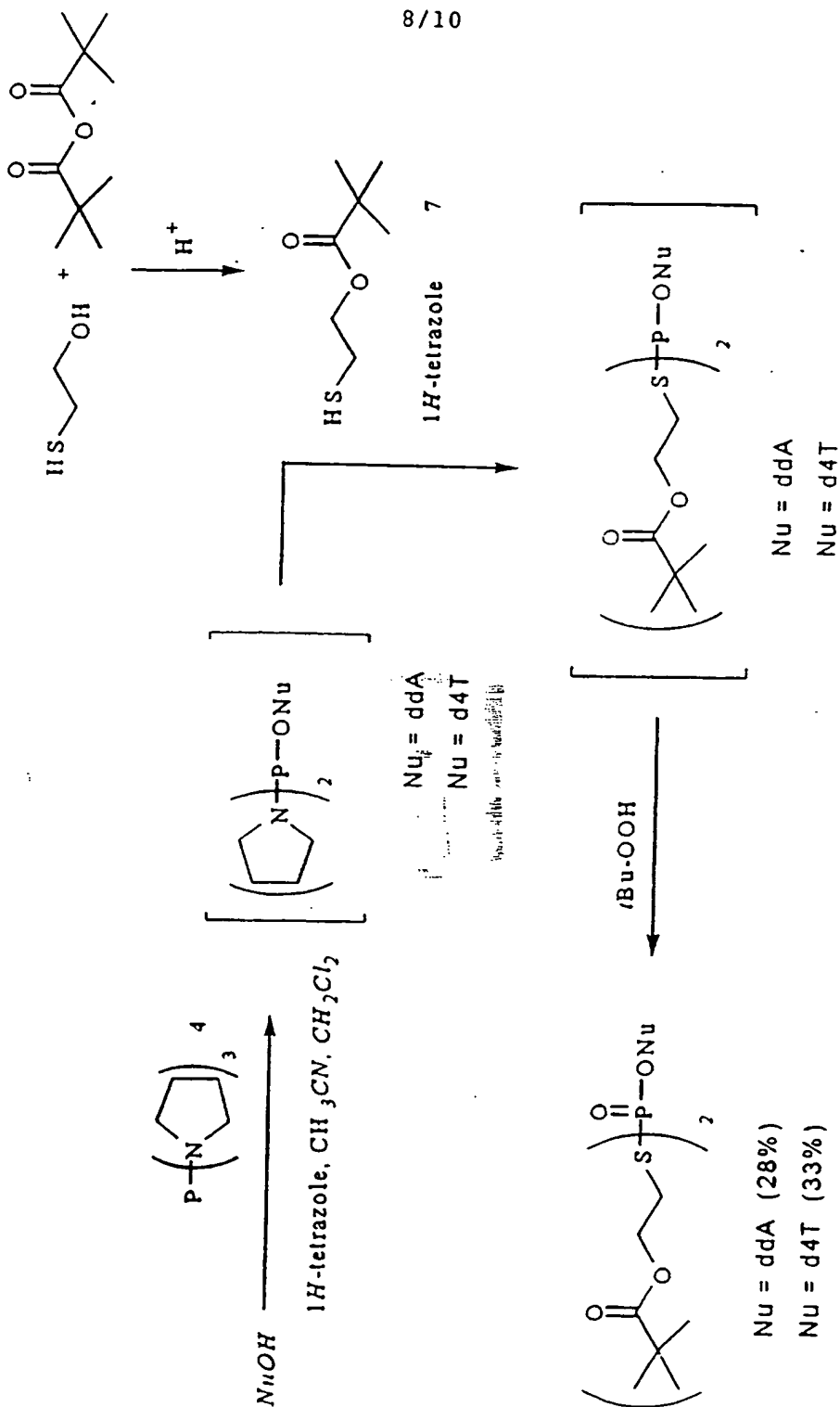


Figure 8

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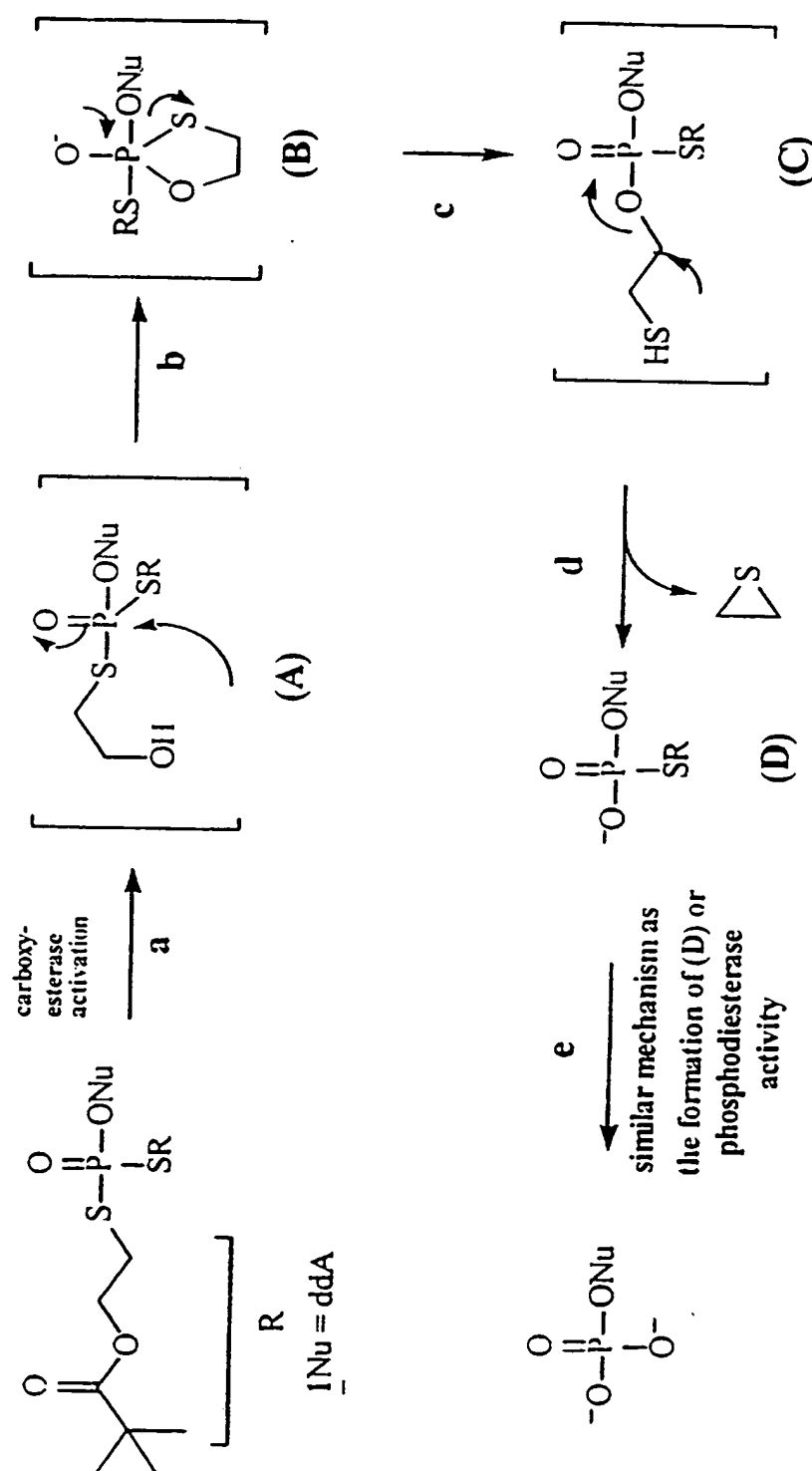
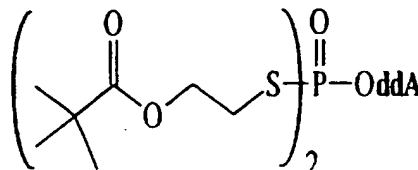


Figure 9

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Anti-HIV activity
of Mononucleoside *S,S'*-bis (*O*-pivaloyl-2-oxyethyl) Phosphorodithiolates

	CEM SS		CEM TK	
	IC ₅₀ (M)	CC ₅₀ (M)	IC ₅₀ (M)	CC ₅₀ (M)
 UA 643	1.2 10 ⁻¹⁰	>10 ⁻⁵	7.7 10 ⁻⁹	10 ⁻⁵
bis(tBuSATE)ddAMP UA 482	1 10 ⁻¹⁰	7.9 10 ⁻⁶	1.3 10 ⁻⁹	1.4 10 ⁻⁶
ddA UA 423	4.9 10 ⁻⁷	>10 ⁻⁴	4.3 10 ⁻⁷	>10 ⁻⁴

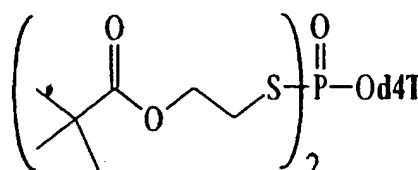
 UA 644	4.4 10 ⁻⁹	>10 ⁻⁵	5.9 10 ⁻⁹	8 10 ⁻⁶
bis(tBuSATE)d4TMP UA 596	3.5 10 ⁻¹³	>10 ⁻⁵	1.7 10 ⁻¹¹	3 10 ⁻⁶
d4T UA 437	4 10 ⁻⁸	>10 ⁻⁴	1.5 10 ⁻⁵	9.2 10 ⁻⁵

Figure 10

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 97/01154

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/10 C07H19/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 93 24510 A (CENTRE NAT RECH SCIENT ;GOSSELIN GILLES (FR); IMBACH JEAN LOUIS (F) 9 December 1993 see the whole document ---	1-14
X,Y	WO 90 08155 A (UNIV TEXAS) 26 July 1990 cited in the application see the whole document ---	1-14
Y	WO 94 26764 A (CENTRE NAT RECH SCIENT ;IMBACH JEAN LOUIS (FR); RAYNER BERNARD (FR) 24 November 1994 see the whole document ---	1-14
Y	WO 96 07392 A (HYBRIDON INC ;IYER RADHAKRISHNAN P (US); YU DONG (US); AGRAWAL SUD) 14 March 1996 see the whole document ---	1-14

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

13 January 1998

Date of mailing of the international search report

30. 01. 98

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Authorized officer

Bardili, W

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IB 97/01154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>J. STELLA ET AL.: "Prodrugs of phosphates, phosphonates and phosphinates" ADVANCED DRUG DELIVERY REVIEWS, vol. 19, 1996, pages 287-310, XP000653348 see page 295, paragraph 4.1.2 see page 301, paragraph 4.1.7 -----</p>	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 97/01154

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